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ANALYSIS OF BIOACTIVE ARYLALKYLAMINES AND THEIR "PRO-DRUGS" IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

by

Ⓒ ADIL J. NAZARALI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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To my wife and to my mother

ABSTRACT

Novel gas-liquid chromatographic methods have been developed which provide a rapid and sensitive means for the analysis of a number of bioactive arylalkylamines and analogues. Aqueous pentafluorobenzoylation or trichloroacetylation was utilized for the analysis of phenylethylamines and anhydrous perfluoroacylation for the analysis of indolealkylamines. The derivatized bioactive amines were subsequently quantitated using a gas-liquid chromatograph with an electron-capture detector. The bioactive amines investigated were β-phenylethylamine (PEA), tryptamine (T) (both endogenous "trace" amines), tranylcypromine (TCP, an MAO inhibitor), and amphetamine (AM, a CNS stimulant).

The N-cyanoethyl, N-chloropropyl, N-*n*-propyl and N-ethoxycarbonyl analogues of the arylalkylamines of interest were studied. These analogues were shown to be metabolized to their respective parent amines *in vivo* to a considerable extent. Rat brain concentrations of the analogue and parent amine were measured at various time intervals after intraperitoneal administration of the analogue of interest. Time-concentration profile data, half-life ($t_{1/2}$) and area under the curve (AUC) measurements from rat brain have been determined for the analogue and parent amine of interest. Results indicate that the time-concentration profile of the parent amines is altered from that of the parent amines obtained after administration of the analogues. The analytical procedure developed provided in most cases simultaneous measurement of the analogue and the parent amine in the same piece of brain tissue. Structures of the final derivatives were confirmed by gas-liquid chromatography-mass spectrometry (GLC-MS).

The analogues were also tested for their inhibitory activity against MAO (monoamine oxidase) enzymes type A and type B. The N-cyanoethyl analogue of TCP was found to be a strong MAO inhibitor *in vivo* and *in vitro*, comparable to the antidepressant TCP. Time studies of TCP and its N-cyanoethyl analogue on the percent inhibition of MAO-A and MAO-B in rat brain were conducted. The biogenic amines 5-hydroxytryptamine, tryptamine and β-phenylethylamine, which are elevated as a result of MAO inhibition, have been quantitated.

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LIST OF ABBREVIATIONS

A	Adrenaline
AA	Acetic anhydride
AUC	Area under the curve
AM	Amphetamine
amu	Atomic mass unit
Anhyd. PFP	Anhydrous pentafluoropropionylation
Aq	Aqueous
Aq. AA	Aqueous acetylation
Aq. PFB	Aqueous pentafluorobenzoylation
Aq. TCA	Aqueous trichloroacetylation
BBB	Blood-brain barrier
Br	Bromine
CAA	Chloroacetic anhydride
CDFAA	Chlorodifluoroacetic anhydride
CE-AM	N-(2-Cyanoethyl)amphetamine
CE-PEA	N-(2-Cyanoethyl)phenylethylamine
CE-T	N-(2-Cyanoethyl)tryptamine
CE-TCP	N-(2-Cyanoethyl)tranylcypromine
CH_3CN	Acetonitrile
CI-MS	Chemical ionization mass spectrometer
Cl	Chlorine
C_{max}	Maximum concentration
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CO_2	Carbon dioxide
CPA	N-(3-Chloropropyl)amphetamine
c.p.m.	Counts per minute
CSF	Cerebrospinal fluid
DA	Dopamine
DADA	3,4-Diacetyldopamine
DAT	O,S-diacetylthiamine
DCAA	Dichloroacetic anhydride
DCPPA	2,4-Dichlorophenoxypropylamine
dec	Decompose
DECT	O,S-Diethoxycarbonylthiamine
DEHPA	Di-(2-ethylhexyl)phosphoric acid
DHPG	3,4-Dihydroxyphenylethylene glycol
DMSO	Dimethylsulphoxide
DOPA	Dihydroxyphenylalanine
DOPAC	Dihydroxyphenylacetic acid
3,4-diOH-CE-AM	3,4-Dihydroxy-N-(2-cyanoethyl)amphetamine
d. water	Distilled water
ECD	Electron-capture detection, detector
ECD-GLC	Electron-capture detector/gas-liquid chromatography
EDTA	Ethylenediaminetetraacetic acid
ETA	Erythrocyte transketolase activity
EthC-AM	N-(Ethoxycarbonyl)amphetamine

EthC-PEA	N-(Ethoxycarbonyl)phenylethylamine
EthC-TCP	N-(Ethoxycarbonyl)tranylcypromine
equiv.	Equivalent
eV	Electron volt
FID	Flame ionization detector, detection
F	Fluorine
g	Gram; gravitional force
GABA	γ-Aminobutyric acid
GLC	Gas-liquid chromatography
GLC-MS	Gas-liquid chromatography-mass spectrometry
GI	Gastrointestinal
h	Hour(s)
HCl	Hydrochloric acid, hydrochloride
$HClO_4$	Perchloric acid
HFB	Heptafluorobutyryl
HFBA	Heptafluorobutyric anhydride
HFBI	Heptafluorobutyrylimidazole
5-HIAA	5-Hydroxyindoleacetic acid
4-OH CE-AM	4-Hydroxy-N-(2-cyanoethyl)amphetamine
4-OH CE-TCP	4-Hydroxy-N-(2-cyanoethyl)tranylcypromine
5-HT	5-Hydroxytryptamine
HVA	Homovanillic acid
I	Iodine
i.d.	Internal diameter
i.m.	Intramuscular
i.p.	Intraperitoneal
KCl	Potassium chloride
kg	Kilogram
$KHCO_3$	Potassium bicarbonate
mol	Mole(s)
m	Metre, milli
MAO	Monoamine oxidase
MAO-A	Monoamine oxidase type A
MAO-B	Monoamine oxidase type B
max	Maximum
MBTFA	N-Methylbis(trifluoroacetamide)
mCi	MilliCurie
ME	Metanephrine
3-MeO-4-OH CE-AM	3-Methoxy,4-hydroxy-N-(2-cyanoethyl)amphetamine
3-MeO-4-OH CE-TCP	3-Methoxy,4-hydroxy-N-(2-cyanoethyl)tranylcypromine
3-MeO-4-OH NPA	3-Methoxy-4-hydroxy-N-*n*-propylamphetamine
MHPG	3-Methoxy-4-hydroxyphenethylene glycol
min	Minute(s); minimum
ml	Millilitre
mm	Millimetre
mmol	Millimole
mmHg	Millimetres of mercury
mol. wt.	Molecular weight

msec	Millisecond(s)
3-MT	3-Methoxytyramine
5-MT	5-Methyltryptamine
m-TA	*meta*-Tyramine
N	Number of subjects
N_2	Nitrogen
NA	Noradrenaline
N-acetylTCP	N-Acetyltranylcypromine
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
ng	Nanogram (10^{-9} g)
NH_4OH	Ammonium hydroxide
^{63}Ni	^{63}Nickel
N-isopropylTCP	N-Isopropyltranylcypromine
nmol	Nanomole(s)
NME	Normetanephrine
NPA	N-*n*-Propylamphetamine
o.d.	Outer diameter
p-Cl PEA	*para*-Chlorophenylethylamine
p-Cl TCP	*para*-Chlorotranylcypromine
Pd-C	Palladium-charcoal
PEA	β-Phenylethylamine
PFB	Pentafluorobenzoyl
PFBA	Pentafluorobenzaldehyde
PFBC	Pentafluorobenzoyl chloride
PFESA	Pentafluoroethanesulfonic anhydride
PFP	Pentafluoropropionyl
PFPA	Pentafluoropropionic anhydride
PFPI	Pentafluoropropionylimidazole
PFPT	Pentafluoropropionyltriazole
pg	Picogram
p-methoxyTCP	*para*-Methoxytranylcypromine
pmol	Picomole(s)
p-OA	*para*-Octopamine
p-OH-AM	*para*-Hydroxyamphetamine
p-OH-NE	*para*-Hydroxynorephedrine
p-OH-NPA	*para*-Hydroxy-N-*n*-propylamphetamine
p-OH-TCP	*para*-Hydroxytranylcypromine
p.s.i.	Pounds per square inch
p-TA	*para*-Tyramine
r.p.m.	Revolutions per minute
SAR	Structure-activity relationship
SCOT	Support coated open tubular
sec	Second(s)
SEM	Standard mean error
SIM	Single ion monitoring
T	Tryptamine
TCA	Trichloroacetyl
TCAA	Trichloroacetic anhydride

TCACl	Trichloroacetyl chloride
TCP	Tranylcypromine
TFA	Trifluoroacetyl
TFAA	Trifluoroacetic anhydride
TFAI	Trifluoroacetylimidazole
TFMSA	Trifluoromethanesulfonic anhydride
TMA	Trimethylamine
t_{max}	Time at which concentration is maximum
TMS	Trimethylsilyl, $(CH_3)_3Si-$
TPD	Thiaminepropyldisulfide
TTFD	Thiaminetetrafurfuryldisulfide
Tyr	Tyrosine
WCOT	Wall coated open tubular

List of Symbols

<	less than
>	greater than
°C	degree centigrade
/	per
%	percent
$t_{1/2}$	elimination half-life
p	probability
df	degrees of freedom

Temperature Reading

All temperature readings in this study are in degrees centrigrade.

OBJECTIVES

Although several methods are available for the gas-liquid chromatographic analysis of the bioactive arylalkylamines, many are lengthy or cumbersome, not sensitive at trace concentrations, and almost all involve preliminary extraction followed by derivatization under anhydrous conditions. Several derivatizing reagents with good GLC properties are available for analysis of arylalkylamines, but a method of analysis has to be considered in its entirety. Poor isolation or separation of the compound of interest is unsatisfactory. Few GLC methods are available for the analysis of secondary arylalkylamines, and almost all such amines are derivatized only when anhydrous conditions and elevated temperatures are used. The phenolic arylalkylamines have been analysed by lengthy procedures employing selective hydrolysis and derivatization under anhydrous conditions to achieve the desired GLC sensitivity and selectivity. A study was therefore initiated to determine whether analytical methods could be found which were rapid, sensitive and selective for the isolation, separation, identification and quantitation of primary, secondary and phenolic arylalkylamines. Arylalkylamines present in a biological milieu with many other endogenous substances had to be quantitatively analysed with these methods. For this purpose halogenated acylating derivatizing reagents were examined for the electron-capture detection/gas-liquid chromatographic (ECD-GLC) analysis of arylalkylamines directly in aqueous biological samples. The methods of analyses developed had to be successfully applied to the quantitation of a number of bioactive arylalkylamines and analogues in rat brain.

A second major objective of this study was to conduct time-concentration profiles in rat brain of the bioactive arylalkylamines and analogues of interest. The half-lives and areas under the curve were calculated to assess the effect of the N-alkyl-substituent on the brain bioavailability of the parent amine.

A final objective of this study was a neurochemical investigation to determine if any of the analogues were active inhibitors of monoamine oxidase (MAO) enzyme type A and type B. The N-cyanoethyl analogue of tranylcypromine was determined to be a strong MAO inhibitor *in vivo* and *in vitro*, comparable to the antidepressant tranylcypromine (TCP). Neurochemical studies were extended and concentrations of the biogenic amines, 5-hydroxytryptamine, tryptamine and β-phenylethylamine were measured. Levels of these substances were found to be significantly elevated as a result of MAO inhibition after administration of N-cyanoethyltranylcypromine. A parallel study was conducted with the clinically used antidepressant TCP.

I. INTRODUCTION

In recent years, much attention has been focussed on bioactive amines structurally related to β-phenylethylamine (PEA) and tryptamine (T). Many are present in foodstuffs (e.g. PEA, T, p-tyramine), are pharmacological agents (e.g. amphetamines, tranylcypromine), and, most importantly, are present in the central nervous system where they are proposed to function as neurotransmitters and as neuromodulators. These bioactive amines (e.g. PEA and T) of the CNS have been implicated in the aetiology of several psychiatric disorders (Dewhurst, 1965, 1968; Fischer and Heller, 1972; Sandler and Reynolds, 1976; Boulton, 1980a; Sabelli et al., 1983) and their low concentrations relative to amines such as dopamine, noradrenaline and 5-hydroxytryptamine have necessitated the development of a number of analytical techniques to better understand the role of these amines in the CNS. Although numerous methods are constantly being developed for the detection of these bioactive amines, many analytical problems remain and there is an evergrowing need for the development of assay methods for sensitive and specific analysis of these substances. This study has demonstrated a number of successful methods of analysis for rapid, specific and sensitive assay of these bioactive amines using gas-liquid chromatography with electron-capture detection (ECD-GLC).

Analytical methods developed were required to conduct time-concentration profiles of the bioactive amines amphetamine, tranylcypromine, PEA and T and their analogues in rat brain. These bioactive amines have been known to be rapidly eliminated from the brain and their N-alkylated analogues were prepared as possible "pro-drugs." It was hoped the pro-drug would provide sustained release of parent bioactive amine in the brain, and, in the case of PEA and T, selectively increase the concentration of the amine in the brain without affecting other endogenous amines.

A. General

A.1 Principles of gas-liquid chromatography (GLC)

The quantitation of low levels of bioactive amines and their metabolites in biological tissues or fluids can be problematic. The "trace" amines tryptamine (T) and β-phenylethylamine (PEA) in particular are found in very low concentrations and other endogenous substances present may cause analytical interferences. Thus, the analysis of bioactive amines poses a significant challenge to the best available instruments and analytical techniques. Most analytical techniques are not selective enough to measure individual substances in a biological sample, and this necessitates the isolation and separation of the desired substance (Olson, 1978). An efficient separation of the substances with minimum interference will significantly improve the selectivity as well as the detector response of the assay method. Gas-liquid chromatography (GLC) techniques are widely used in modern analytical laboratories and allow efficient separation, quantitation and identification of individual components in a sample mixture. Essentially, GLC is a system whereby a carrier gas (mobile phase) is allowed to pass through a column containing small particles (solid support) coated with a thin film of nonvolatile liquid (stationary liquid phase). When a sample mixture is introduced into the column at a desired temperature, it volatilizes and each component partitions between the mobile gas phase and the stationary liquid phase. As there is a continuous flow of the carrier gas, equilibrium is not established and each component moves along the column. The rate at which each component moves through the column will depend on the extent of its distribution between the two phases. The components separate from each other according to their partition coefficients between the stationary liquid phase and the carrier gas. The GLC oven, where the column is housed, is usually operated at a temperature range of 20-300° (Coutts and Baker, 1982). The temperature setting will depend on the volatility of the compounds of interest and the stability of the stationary liquid phase. The column temperature can be kept constant, allowing an isothermal separation of components, or the temperature can be programmed, whereby the column temperature increases at a preset rate (McCullough and Aue, 1973). Only the low boiling components will pass through with any significant velocity at the initial column temperature and the high boiling components are trapped at the beginning of the column until the oven temperature rises. Hence temperature programming allows greater resolution and shorter analysis time when analysing a sample mixture containing a wide range of components (Baker et al., 1982a). When each component elutes from the column it meets with the detector and sets up an electrical signal which is displayed as a peak on the chart recorder. The retention time of the peak is measured from the point of injection to the apex of the peak. It is a characteristic fingerprint, but not necessarily unique, for each component under the experimental conditions (Brenner and Olson, 1967).

A.2 Columns for GLC

The column is an important part of the gas chromatograph since this is where the individual components are separated. It is composed of a column tubing, a solid support and the stationary liquid phase. The column tubing is generally made of glass, but stainless steel, copper, nickel, aluminium and glass-lined metal columns have also been used (Fenimore et al., 1977; Coutts and Baker, 1982). Glass is frequently the column material of choice because of its inert nature and the ease with which column packings can be visually checked initially and during the lifetime of the column. However, the flexible and strong fused silica columns are gaining preference over the more fragile glass columns. The column itself generally is not involved in the chromatographic process except in an adverse manner. Heated metal columns are prone to react with organic compounds. Copper, for instance, reacts with amines, terpenes and steroids (McNair and Bonelli, 1969; Baker et al., 1982a).

The solid support of a packed column is inert and provides a large surface area for the liquid phase and is often made of diatomaceous earth (kieselguhr). There are basically two types of support material with different physical properties. Chromosorb® W (White) is a low density support and can retain as much as 30% stationary liquid phase (Coutts and Baker, 1982). Chromosorb® P (Pink) is mechanically stronger but retains much lower amounts of stationary phase (McNair and Bonelli, 1969). Polymer beads (Porapak®) have also been used as packing material and were first introduced by Hollis (1966). The polymer beads serve as both stationary liquid phase and solid support. Separation on Porapak® occurs by a combination of partition and adsorption (Zweig and Sharma, 1972).

The stationary liquid phases can be classified according to their polar nature. The polymers of polyethylene glycol (carbowax) are polar and polymers of dimethylsilicone (SE-30, OV-101) are non-polar. Semipolar phenylmethylsilicone polymers (OV-17) are also used. The choice of a liquid phase from the several hundred available is an empirical process. Generally, separations are best achieved by matching solute and liquid types. Polar liquid phases retain polar solutes whereas non-polar liquid phases retain non-polar solutes (Mitchard, 1978). The liquid phase is then the active ingredient of the column where solutes with strong affinity for the liquid phase emerge last and solutes with a weaker affinity emerge first. In drug separation studies columns are lightly loaded with liquid phase, since the concentration of the liquid phase will influence the resolution and the retention time of the solute. The usual concentration of the liquid phase on a solid support is 1 to 5% w/w (Moffat, 1975; Coutts and Baker, 1982).

The size of the columns used can vary. A typical packed column is 1 to 2 m in length and 2 to 4 mm in internal diameter. It is relatively robust and inexpensive and allows the analyst a flexibility in preparing a large number of liquid phase concentrations on various solid supports. Capillary columns of 10 to 75 m in length and 0.25 to 0.50 mm in internal diameter are commonly used in trace analysis. There are two principal types of capillary columns in use. The support-coated open tubular (SCOT) capillary columns have a solid support with a thin film of liquid phase and can generally withstand higher sample input. Some capillary columns have only a thin coating of liquid phase in the inner wall; these are the wall-coated open tubular (WCOT) columns (Coutts and Baker, 1982).

Generally, the resolution of solutes obtained with capillary columns is superior to that achieved with packed columns. A number of columns, both packed and capillary, have been employed in the analysis of bioactive amines. A glass capillary column (10 m, WCOT SP2100) has been used in the simultaneous analysis of β-phenylethylamine (PEA), m-tyramine (m-TA), p-tyramine (p-TA), p-octopamine (p-OA), normetanephrine (NME), and 3-methoxytyramine (3-MT) (Fig. 1) (LeGatt et al., 1981). A packed column, 3% OV-17, employed by the same authors separated the amines but proved to be much less sensitive. Calverley et al. (1980) used a WCOT, OV-101 glass capillary column for simultaneous analysis of tryptamine (T) and 5-hydroxytryptamine (5-HT).

A.3 Electron-capture detection (ECD)

The electron-capture detector is among the most sensitive measuring devices in GLC (Sevcik, 1976; Olson, 1978; Baker et al., 1981). It is known to detect as little as one picogram (10^{-9} g) of some compounds in a 1 μl injection. Lovecock and Lipsky (1960) were the first to introduce ECD to gas chromatography. The mechanism of ECD depends on the electron-capturing ability of the sample compound. Electrophores such as halogen, nitro, hydroxy and carbonyl groups exhibit selective response for ECD (Baker et al., 1981). The electron-capture detector employs a radioactive isotope, most commonly nickel-63 (^{63}Ni). This radioactive isotope during its normal decay process releases β-particles which in turn collide with the carrier gas molecules (argon/methane 90:10) and produce a large number of electrons. These electrons are collected at the anode and produce a small measurable "standing current." As the electrophores elute from the gas-chromatographic column and meet with the detector, they capture electrons to form negatively charged ions. This results in a depletion of electrons and a decrease

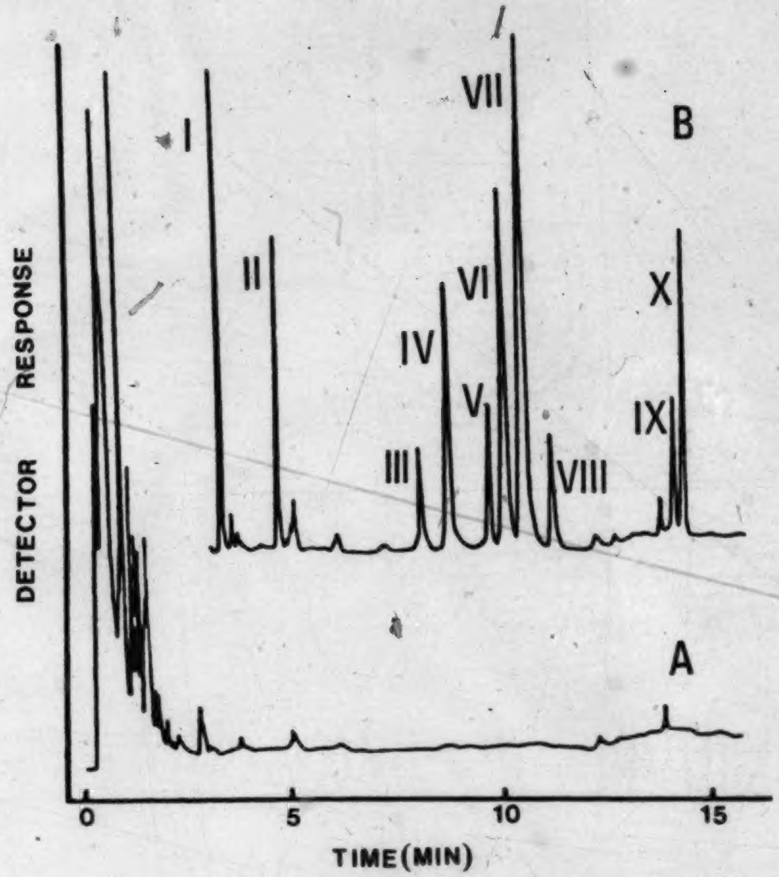


Fig. 1. GC traces of (A) hydrochloric acid blank; (B) standard prepared with 500 ng of each of the amines. Peaks are derivatives of: I, benzylamine; II, β-phenylethylamine; III, 3-phenylpropylamine; IV, m-tyramine; V, tranylcypromine; VI, p-tyramine; VII, 2-(4-chlorophenyl)ethylamine; VIII, p-octopamine; IX, normetanephrine; X, 3-methoxytyramine on an SP2100 glass capillary column (adapted from LeGatt *et al.*, 1981).

in the "standing current," which is recorded as a peak on the chart recorder after amplification. When the electrophore leaves the detector the current returns to the original value of the standing current.

Electrons from the cell are collected by three basic methods: direct current (D.C.), pulsed, and linear. The D.C. method applies a constant voltage to the electrodes, collecting electrons continuously to produce a steady current. This method suffers from a number of disadvantages. Sensitivity is poor since the negative ions formed by electron capture will be collected at the anode, and the reduction in current is less than if only the electrons were collected. The negative ions can collect on the electrode and contaminate the electrode surface. These factors lead to a non-linear response of the detector.

The pulsed method applies the collecting voltage in pulses of one microsecond in duration at intervals of 5 to 150 microseconds. Since the pulse voltage is applied for such a short time, it does not give the larger, heavier negative ions time to collect on the anode, allowing the much lighter free electrons to be collected rapidly. Hence only the electrons are collected, which enhances the sensitivity of the detector compared with the D.C. method. The linear dynamic range of the pulse method is poor, and only after careful adjustment of the pulsed voltage parameters will the linear range be about 1000.

The linear method is a modified version of the pulsed method (Maggs et al., 1971). The pulsed voltage with ECD electronics is set up so that a constant cell current is produced. When an electrophoric species meets with the detector and captures the free electrons, this leads to a drop in the standing current. With the use of electronics the pulse frequency is automatically increased (to compensate for the loss of the electrons) and maintains a constant standing current. Thus an increase in pulse frequency corresponds to an increase in the concentration of the electrophoric species. With this type of detector, it is possible to obtain linear ranges exceeding 10,000.

B. Sample Isolation from Brain Tissue Prior to Chromatography

A pure compound in its crystalline form is in most cases simple and straightforward to analyse by chromatographic means. However, when present in a biological medium with numerous other endogenous substances, the compound is not so easy to analyse. Interfering substances can overload the column packing, thereby reducing its resolving capabilities and/or saturating the detector, which will raise the noise level and threshold detection limit. Thus, in order to have efficient resolution and maximum detection capability, one must perform purification procedures prior to chromatography. The compound of interest, when encapsulated in a biological matrix such as tissue, cells, or mycelia, has to be isolated from its matrix and this is usually done by homogenizing and extracting.

B.1 Tissue grinders

Brain tissue is among the softest and most easily disrupted tissue (Ko and Petzold, 1978). The Potter-Elvehjem homogenizer is the most commonly used tissue grinder (Ko and Petzold, 1978; Martin and Baker, 1977). Brain tissue is homogenized in special homogenizing glass tubes in a small amount of extracting medium (e.g. perchloric acid, HCl-methanol, and ethanol). The tissue is frozen with solid carbon dioxide or liquid nitrogen prior to grinding to prevent degradation or metabolism. The supernatant is obtained by centrifugation (e.g. 10,000 x g at 0-4°) and discarding the precipitated protein.

B.2 Ultrasonic method

Ultrasonic waves which generate collapsing bubbles selectively break covalent bonds of polymers with molecular weights greater than 0.5 million (Ko and Petzold, 1978). Thus the ultrasonic waves rupture the cell walls and leave the drug or amine molecule intact. In Table I, various tissues are listed in ascending order with regard to difficulty in disrupting the tissue. Brain tissue is shown to be the most easily disrupted.

A major disadvantage with the use of ultrasonic disruption is that intense localized heat is generated when the microscopic bubbles collapse. This in turn induces free radical formation and leads to denaturation of enzymes, hydrolysis of esters, and oxidation of unsaturated compounds. Addition of a small quantity of a volatile liquid heat-sink like methanol or acetone will dissipate the heat. Degassing the water or saturating it with carbon dioxide or hydrogen can reduce free radical formation (Ko and Petzold, 1975).

B.3 Extraction of amines from brain tissue

B.3.1 Resins, adsorbents and molecular sieves

The liquid ion-pairing reagent di-(2-ethylhexyl)phosphoric acid (DEHPA) has been used efficiently for isolation of amines (Temple and Gillespie, 1966; Martin and Ansell, 1973; Nelson et al., 1979; Baker et al., 1980; Hampson et al., 1984a). It has an added advantage of being a

Table I. Tissue disruption in ascending order of difficulty (adapted from Ko and Petzold, 1978).

1	Brain
2	Mucous
3	Tissue culture
4	Fat
5	Faeces
6	Necrotic tissue
7	Tumour cells
8	Liver
9	Bladder
10	Aorta
11	Kidney
12	Lung
13	Fibrin
14	Skin
15	Muscle
16	Bone
17	Heart muscle
18	Green leaves
19	Hair
20	Renal papilla

liquid reagent which allows efficient mixing of biological sample and resin and isolation of amines.

A number of methods for isolation of biogenic amines on Sephadex columns have been published (Contractor, 1966; Renaud et al., 1974; Boireau et al., 1976; Westerink and Korf, 1976, 1977; Westerink and Mulder, 1981; Westerink, 1984). All of the above methods utilized Pasteur pipettes or glass tubes for columns and most used a glass wool plug. Feenstra et al. (1982) used Sephadex G10 columns for isolation of dopamine (DA) and analogues. Recently Westerink (1984) employed similar Sephadex G10 columns for isolation of tyrosine (Tyr), 3,4-dihydroxyphenylalanine (DOPA), noradrenaline (NA), DA, 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylethylene glycol (DHPG), and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). Earley and Leonard (1978) introduced disposable Bio-Rad columns packed with Sephadex G10 with microporous filters for simultaneous isolation of NA, DA, 5-HT, and 5-HIAA.

Many other substances, e.g. XAD-2 resin (Kullberg and Gorodetzky, 1974; Delbeke and Debackere, 1977; Artigas and Gelpi, 1979), charcoal (Meloa and Vanko, 1974), Amberlyst (Martin and Baker, 1977), Porapak Q (Snodgrass and Horn, 1973), and Dowex (Boulton et al., 1976) have been used for isolation of bioactive amines and drugs.

A technique employing aluminium oxide and Amberlite CG50 columns in sequence for isolation of a number of amines has also been used (Karasawa et al., 1975; Calverley et al., 1981b). Shaw (1938) was the first to describe adsorption of catecholamines onto alumina. Since that time, alumina has been employed extensively for the isolation of catecholamines (e.g. Anton and Sayre, 1968; Arnold and Ford, 1973; Todoriki et al., 1983). The adsorbed catecholamines can be eluted from the alumina with a small amount of HCl, methanol-HCl, or ethanol-acetic acid mixture. Boric acid gel has also been used to provide high recoveries of the catecholamines from aqueous solutions (Higa et al., 1977). Molecular sieves such as the millipore filters have not had wide use. The millipore filters were initially developed for separating bacteria from solution and still play an important role in purification. The filters were eventually applied to separate macromolecules from biological fluids; however, they tend to absorb drug molecules and related substances present in low concentrations (Mitchard, 1978).

B.3.2 Solvent extraction

The principle that the non-ionized form of a molecule is more soluble in an organic solvent than the ionized polar form can be applied in the isolation of basic amines. Impurities are removed from the primary extract by using both acid and base extraction. A generalized scheme for isolation of acid, base or neutral molecules is illustrated in Figure 2 (Mitchard, 1978). The sequence of acid or base extraction can be alternated. This would then be termed "back-extraction."

Amines in a basic pH environment exist in a non-ionized form, and generally extraction into an organic solvent works well. However, the presence of other functional attachments such as phenolic hydroxyl groups can alter the pK_a and other inherent properties. A phenolic amine would then be amphoteric and exist as a Zwitterion. This would not allow adequate recoveries into the organic solvent. To get around this problem, direct aqueous acylation of the biological sample can be

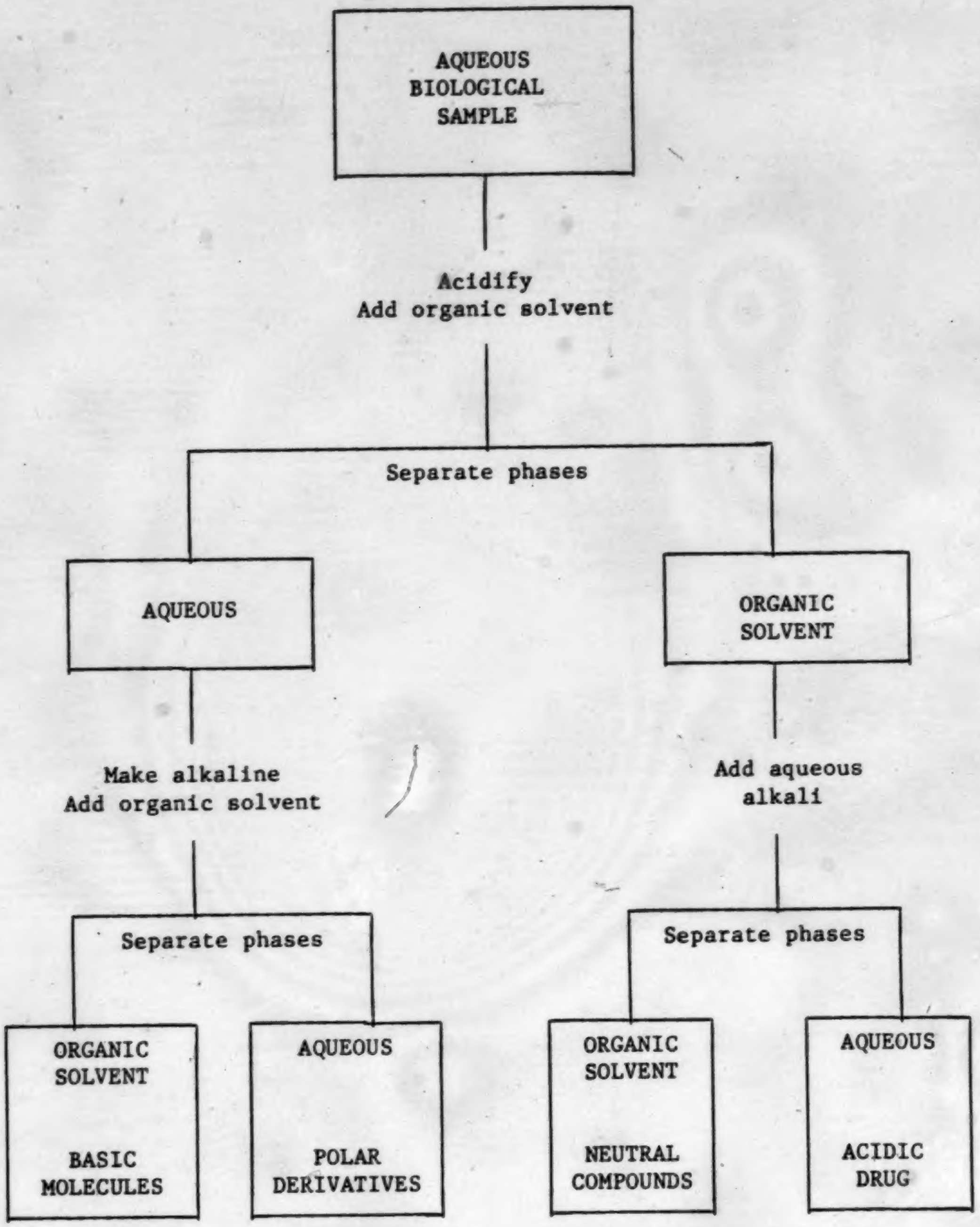

AQUEOUS BIOLOGICAL SAMPLE
Acidify
Add organic solvent
Separate phases
AQUEOUS
ORGANIC SOLVENT
Make alkaline
Add organic solvent
Add aqueous alkali
Separate phases
Separate phases
ORGANIC SOLVENT
BASIC MOLECULES
AQUEOUS
POLAR DERIVATIVES
ORGANIC SOLVENT
NEUTRAL COMPOUNDS
AQUEOUS
ACIDIC DRUG

Fig. 2. Acid-base solvent extraction (adapted from Mitchard, 1978) of a molecule.

employed. This renders the molecule neutral and non-polar, facilitating efficient extraction (Coutts et al., 1980). This topic is further discussed in Section C (Chemical derivatization in GLC).

C. Chemical Derivatization in GLC

Gas-liquid chromatography has played a very valuable role in chemical analysis, and has had a significant impact on a variety of scientific fields, e.g. the clinical sciences, pharmacology, toxicology, environmental science and forensic science. Although many compounds can be analysed on GLC, there still remains a great many that cannot. Much effort has been directed into finding ways of making derivatives of these compounds in order to obtain a successful GLC analysis. The compound can be converted into a stable volatile derivative to achieve optimum sensitivity, specificity or selectivity with efficient elution and separation. A number of excellent review papers and books discuss the subject of derivatization reactions for GLC (Drozd, 1975; Ahuja, 1976; Perry and Feit, 1978; Blau and King, 1978; Knapp, 1979).

C.1 Principles for employing chemical derivatization in GLC

An important consideration for employing chemical derivatization is to synthesize a volatile derivative of an essentially non-volatile compound to impart the desired gas chromatographic properties. Polar molecules with N-H, O-H, or S-H groups possess strong intermolecular hydrogen bonding and will have low volatility. Acylation, alkylation or silylation of the polar groups will significantly increase volatility (Knapp, 1979).

However, derivatization can also be used to decrease volatility. Excessive volatility can pose a problem in the analysis of small molecular weight compounds. In this case, derivatization can be used to yield less volatile derivatives for an efficient separation and identification.

The adsorption of polar molecules on the active sites of the column solid support will result in long retention times and asymmetrical peaks. Derivatization will reduce adsorption effects and significantly improve peak shape. Stability can also be enhanced with derivatization, leading to improved chromatographic performance.

Thus, chemical derivatization is generally employed to increase volatility and stability, to improve chromatographic performance by removing polar groups, to decrease excessive volatility, to enhance extraction efficiency (e.g. aqueous acetylation of phenolic amines) discussed in Section C.4 (Acylation of amines in aqueous medium), and/or finally to confer improved detectability, discussed in Section C.2 (Derivatization for improved detectability by ECD).

C.2 Derivatization for improved detectability by ECD

Most compounds are not sensitive to ECD in their original native form, but when converted to ECD-sensitive derivatives their detectability is enhanced considerably. Trace level analysis can then be performed with ease using the sensitive ECD technique. Many types of derivatives have been used for ECD-GLC analysis (Poole and Morgan, 1975; Knapp, 1979). Halogenated acyl groups are among the most commonly used derivatives. Generally, substitution with halogen increases the electron-capturing ability of the compound in the order of I > Br > Cl > F.

Usually increasing the number of electron-capturing moieties on a single carbon increases the sensitivity several-fold in a non-linear manner. The nitro group has about the same electron-capturing ability as a chloro group. Electron-donating substituents on the aromatic ring will reduce electron affinity and therefore ECD-GLC sensitivity, whereas electron-withdrawing substituents will enhance sensitivity.

The carbonyl group (C=O) was initially proposed to be the focus of electron capture (Landowne and Lipsky, 1963). However, Clarke *et al.* (1967) found that heptafluorobutyryl derivatives were more sensitive than trifluoroacetyl derivatives and reasoned that the halogenated alkyl chain was the site of electron capture. Subsequent studies by Moffat *et al.* (1972) and Matin and Rowland (1972) with ECD-sensitive derivatives of amines indicated that the presence of a double bond between nitrogen or oxygen and the carbon atom (C=O, C=N) adjacent to the halogenated group was a structural requisite for good electron-capturing properties.

C.3 Halogenated acyl derivatives employed in ECD-GLC analysis of amines

Halogenated acyl derivatives are among the most popular derivatives for ECD chromatography. A number of halogenated acyl derivatizing reagents have been employed (Fig. 3). Of these, trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), and heptafluorobutyric anhydride (HFBA) are used most frequently.

Most acylating reagents can react not only with amines, but also with phenol and alcohol substituents in a non-aqueous medium. This is a significant advantage in the analysis of biogenic amines present in the CNS, since many (e.g. NA, DA, 5-HT, *p*-TA, *p*-OA) possess hydroxyl func-

$(C_nF_{2n+1}CO)_2O$

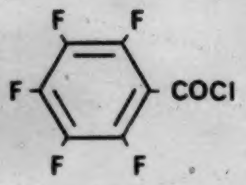
TFAA, n = 1
PFPA, n = 2
HFBA, n = 3

$(ClCR_2CO)_2O$

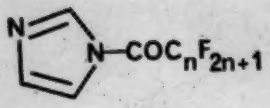
CAA, R = H
CDFAA, R = F
TCAA, R = Cl

$(C_nF_{2n+1}SO_2)_2O$

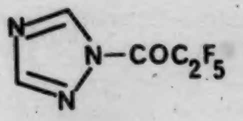
TFMSA, n = 1
PFESA, n = 2



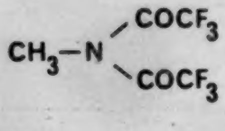
PFBC


N
N—COC$_n$F$_{2n+1}$

TFAI, n = 1
PFPI, n = 2
HFBI, n = 3



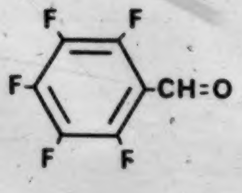
PFPT


COCF$_3$
CH$_3$—N
COCF$_3$

MBTFA

CCl_3COCl

TCACl



PFBA

Fig. 3. Structures of some halogen-containing acylating reagents and related derivatizing reagents used in ECD-GLC. (For full explanation of abbreviations, see List of Abbreviations, pp. xix-xxii .)

tional groups. Thus derivatization of all the polar groups of the molecule will increase sensitivity and improve chromatographic performance, especially since the O-perfluoroacyl derivatives are generally more strongly electron-capturing than the corresponding amine derivatives (Clarke et al., 1966). For most derivatization schemes, TFAA, PFPA and HFBA can be used interchangeably, but they may differ in the degree of reactivity, sensitivity, and volatility imparted and these characteristics can vary with the nature of the molecule being derivatized. Hence the choice of any one derivative will depend on the analytical priorities of the chromatographer, and this is illustrated with the numerous examples given below.

Clarke et al. (1966) compared the ECD sensitivity of TFA, PFP, HFB and chloroacetyl derivatives of a number of amines. The order of sensitivity for the derivatives of the non-hydroxylated amines was HFB > PFP > chloroacetyl > TFA. The N,O-di-TFA derivative of tyramine was reported to be 10,000 times more sensitive to ECD than the mono-N-TFA derivative. The N-TFA derivative in general was relatively insensitive to ECD. However, TFA derivatives have been used successfully in the ECD-GLC analysis of metanephrine (ME), NME, p-TA, 3-MT, p-OA, T, synephrine, and 5-HT (Sen, 1969; Bertani et al., 1970). Martin and Ansell (1973) quantitated the catecholamines NA and DA and the indoleamine 5-HT in rat brain using TFAA as the derivatizing reagent. The catecholamines were isolated on alumina and separated from the aqueous phase with DEHPA, whereas 5-HT was extracted with 20% v/v n-butanol in diethyl ether. The trifluoroacetyl derivatives were reported to be formed with ease and were relatively stable under anhydrous conditions. Several other researchers have analysed the catecholamines NA, DA and adrenaline

(A) in urine, tissues and serum with ECD-GLC after derivatizing with TFAA (Clarke _et al._, 1967; Kwai and Tamura, 1968a, 1968b; Imai _et al._, 1971). The reagent TFAA has also been used under anhydrous conditions for the ECD-GLC analysis of _p_-hydroxyamphetamine (_p_-OH-AM) and _p_-hydroxynorephedrine (_p_-OH-NE) in rat brain (Belvedere _et al._, 1973). Joseph (1978) employed TFAA for the ECD-GLC analysis of kynurenine in urine, plasma, brain and CSF. Anggard and Sedvall (1969) compared the TFA, PFP and HFB derivatives of ME, NME, and 3-MT. They reported the PFP and HFB derivatives to be more stable with better ECD response than the TFA derivatives. Ko _et al._ (1974) prepared PFP, HFB and TFA derivatives of DA and found PFP derivatives to be more stable than TFA or HFB derivatives. Similarly, Arnold and Ford (1973) prepared PFP and TFA derivatives of a number of biogenic amines and related compounds (DA, NA, A, DOPA and DOPAC). The PFP derivatives were reported to be more stable than the TFA derivatives. Their method was subsequently adopted by Freed _et al._ (1977) for the simultaneous analysis of DA, NA and their α-methyl analogues by CI-MS.

Wong _et al._ (1973) analyzed a number of biogenic amines as their PFP derivatives. The derivatization reaction was in presence of heat (65°) for 1 h. Analysis was performed on 10% SE-24 column at an isothermal temperature of 200° with an ECD-GLC. Many researchers have adopted this general method for preparing PFP and TFA derivatives of the catecholamine metabolite 3-methoxy-4-hydroxyphenethylene glycol (MHPG). Most of the methods developed vary only in degree from Wong's method, utilizing varying proportions of anhydride and ethyl acetate or varying reaction times and temperatures (Dekirmenjian and Maas, 1970; Wilk _et al._, 1971; Gordon and Oliver, 1971; Karoum _et al._, 1971; Bond, 1972;

Braestrup, 1973; and Fellows et al., 1975). Karoum et al. (1972) prepared PFP derivatives of 13 biogenic amines and compared their chromatographic performance with HFB and TMS,HFB derivatives. The PFP derivatives were reported to give the highest GLC resolution. Optimum derivative conditions and GLC behaviour of PFP derivatives of some 28 catecholamines and related compounds (including the metabolites ME and NME) were reported by Gelpi and his colleagues (1974). Baker et al. (1982b) employed PFP derivatives in the analysis of p-chlorophenylethylamine (p-Cl-PEA) in brains of rats treated with a monoamine oxidase inhibitor (pargyline, 60 mg/kg i.p.) and varying doses of p-chlorophenylalanine. The assay procedure employed direct extraction with an equal volume of ethyl acetate after basifying and buffering an aliquot of the supernatant. The ethyl acetate layer was retained and dried under a stream of nitrogen (N_2) at room temperature. Ethyl acetate (20 µl) and PFPA (100 µl) were added to the dried residue. Derivatization was allowed to proceed to completion for 60 min at 60°. Analysis was performed with ECD-GLC equipped with a 6 ft glass column packed with 3% OV-17.

Heptafluorobutyric anhydride (HFBA) has been employed in the ECD-GLC analysis of amphetamine (AM), methamphetamine and fenfluramine in blood after basic extraction into pentane (Bruce and Maynard, 1969). Cummings and Fourier (1969) measured pseudoephedrine in plasma after preparing HFB derivatives. The ECD response was reported to be linear from 0-1000 ng for HFB derivatives of pseudoephedrine, ephedrine and norephedrine. Fujihara et al. (1983) prepared HFB derivatives of polyamines after first isolating the polyamines from blood by means of activated Permutit. Heptafluorobutyric anhydride (50 µl) in 200 µl acetonitrile were used and derivatization was allowed to proceed for 10 min at

65°. Derivatization with HFBA has also been employed for the ECD-GLC analysis of various drug molecules (Sioufi et al., 1983a,b; Jeanniot et al., 1983).

Perfluoroacyl imidazoles are important alternative acylating reagents. Trifluoroacetyl imidazole (TFAI), pentafluoropropionyl imidazole (PFPI), and heptafluorobutyryl imidazole (HFBI) reagents have an advantage in that they provide a non-acidic environment, since a basic leaving group is formed during derivatization. Hence the perfluoroacylating imidazoles would be suitable, for example, for analysis of acid-sensitive compounds which are prone to dehydration in an acid medium (Sugiura and Hirano, 1974). Although the perfluoroacylating imidazoles can have an advantage over the use of anhydrides, they do pose a problem. They are highly viscous and therefore difficult to draw up in a syringe and to remove at the end of the reaction (Baker et al., 1981). It is important to remove the excess reagent since it is likely to depress the standing current of the ECD, which would give false quantitation of eluting compounds. The excess acylating reagent, if not removed, can also cause problems such as irreversible change in the GLC column, corrosion within the GLC or GLC-MS system, or re-derivatization of non-volatile components to give "ghost" peaks (Blau and King, 1978). Therefore, the acid anhydrides, which are generally often easier to remove, are more popular. However, a number of researchers do use perfluoroacylating imidazole reagents. Vessman et al. (1969) employed HFBI for the ECD-GLC analysis of indoleamines, preparing HFB derivatives of T, 5-HT, tryptophol and several N- and O-alkyl analogues. The procedure requires heat at 80° for 2-3 h. Degen et al. (1972) quantitated melatonin in rat pineals. The method employed HFBI to quantitate melatonin to 35 pg using ECD-GLC. Analyses of T and 5-HT have been reported with ECD-GLC. Heptafluorobutyryl derivatives have been prepared by reaction with HFBI at 80° for 1 h (Benington *et al.*, 1975; Christian *et al.*, 1975). Pentafluoropropionyl triazole (PFPT), a derivatizing reagent whose use has not often been reported, has been used for the analysis of a number of biogenic amines and metabolites. In this case triazole is the basic leaving group, providing a non-acid environment (Miyazaki *et al.*, 1974).

The acyl halides pentafluorobenzoyl chloride (PFBC) and trichloroacetyl chloride (TCACl) are highly reactive and used in the acylation of compounds that are difficult to acylate, such as amides or compounds with steric hindrance (Blau and King, 1978). The only disadvantage to using these acyl halides is the release of the halogen acid during derivatizing reaction. A base is then required to neutralize the acid. The removal of the excess acyl halide and the halide salt formed with the base may require additional separating steps in the assay. The anhydrides are generally easier to remove after completion of derivatizing reaction and give cleaner chromatographic spectra. However, these reagents (PFBC and TCACl) often produce highly sensitive derivatives compared to the anhydrides. This is illustrated in some examples below. Wilkinson (1970) prepared TFA, PFP, HFB, pentadecafluorooctanamide and PFB derivatives of AM, PEA and ephedrine. The PFB derivatives were reported to give the highest ECD responses in all cases. Moffat *et al.* (1972) prepared a number of pentafluorophenyl derivatives of PEA and N-methylphenylethylamine and measured their ECD sensitivities. Pentafluorobenzoyl chloride was reported to be the best reagent for the assay of the secondary amines, and the choice for the derivatization of primary amine was either PFBC or pentafluorobenzaldehyde (PFBA). Derivatization was performed under anhydrous conditions in the presence of heat (60°) for 1 h. In order to formulate a hypothesis for the electron-capture mechanism, Matin and Rowland (1972) employed a variety of ECD-sensitive derivatizing agents for the analysis of primary and secondary amines. It was found that PFBC was suitable for primary as well as some secondary amines; however, when the primary and secondary amines were present together, the latter could cause interference with the former in the chromatogram. In such a case HFBA was preferred. In a comparatively recent study, Midha _et al._ (1979a) prepared the N-PFB, N-PFP, N-HFB and N-TFA derivatives of a number of ring-substituted phenylisopropylamines and analysed them with ECD-GLC. The electron-capture response of the Ni^{63} detector to N-PFB derivatives was found to be 10 to 15 times greater than to N-HFB and N-PFP derivatives, and 100 to 150 times greater than to N-TFA derivatives. This work agrees well with that of Cummings (1971) who reported that PFBC rendered much greater sensitivity to a number of amines than did HFBA. A survey of the literature appears to indicate that, of the derivatizing reagents commonly available, PFBC confers the highest ECD sensitivity for amines (Moffat _et al._, 1972) and also for phenols (McCallum and Armstrong, 1973). The PFB derivatives are prepared under anhydrous conditions, are comparatively easy to make, and are stable with good chromatographic performance. However, it seems that researchers have been slow in exploiting the excellent properties of PFBC in the analysis of biogenic amines. Recent publications in the area of amine analysis with ECD-GLC indicates that PFBC use is indeed becoming more prevalent. Reynolds _et al._ (1978) employed PFBC for the analysis of AM and PEA in post-mortem Parkinsonian brain after

(-)-deprenyl administration. Midha et al. (1979b) prepared PFB derivatives for a simultaneous analysis of norfenfluramine and fenfluramine in human plasma and urine. The derivatization reaction proceeded for 30 min at 65°. Pentafluorobenzoyl derivatives of the catecholamines NA and A have also been prepared by Bock and Waser (1981) for ECD-GLC analysis.

In all the above examples, PFBC was employed to prepare derivatives under anhydrous conditions, and, in most cases, in the presence of heat. However, recently Cristofoli et al. (1982b) prepared AM derivatives with PFBC in an aqueous environment. This is discussed in Section C.4 (Acylation of amines in aqueous medium).

The acylating reagent trichloroacetyl chloride (TCACl) has been used for the analysis of AM and phenmetrazine in plasma and urine. The derivatization reaction proceeded for 30 min in presence of heat (40°). The method allowed measurements down to 1 ng/ml with ECD-GLC (Anggard et al., 1970). This reagent has also been employed by other researchers for sensitive ECD-GLC analysis. Noonan et al. (1969) used TCACl for analysis of AM in urine, plasma and saliva of horses, and Baselt et al. (1977b) for a tranylcypromine (TCP) assay in serum and urine. A series of halogenated acyl derivatives of amines was prepared by Anggard and Hankey (1969). Trichloroacetyl chloride was reported to give greater ECD response than TFA, PFP or HFB derivatives and was second only to PFBC.

Numerous other halogen-containing derivatizing reagents are available commercially but have not been used in the analysis of bioactive amines to any significant extent. N-Methyl-bis(trifluoroacetamide) is a trifluoroacylating reagent developed by Donike (1973). It was reported to acylate primary and secondary amines but hydroxyl groups were not as reactive. Chloroacetic anhydride, a reagent that has been available but not used to any great extent, has been reported to acylate phenols in aqueous medium (Argauer, 1968). Similar observations were made by Coutts et al. (1980a) with the extraction of hydroxylated amines from aqueous solution with chloroacetic anhydride (CAA) and dichloroacetic anhydride (DCAA). Dehennin et al. (1972) prepared chloroacetyl derivatives of hydroxylated compounds and reported good ECD sensitivity. However, the corresponding amine derivatives have poor ECD sensitivity (Anggard and Hankey, 1969). Chlorodifluoroacetic anhydride (CDFAA) is another reagent that has not been extensively used. Cockerill et al. (1975) employed CDFAA in presence of heat (50° for 30 min) for the ECD-GLC analysis of the drug 1-(2-phenyladamant-1-yl)-2-methylaminopropane in blood plasma and urine. Preliminary studies by Baker et al. (1981) indicate that CDFAA yields sensitive derivatives of catecholamines under anhydrous conditions.

Recently, trichloroacetic anhydride (TCAA) has been employed for simultaneous analysis of PEA and TCP in rat brain after preparing derivatives in aqueous medium (Baker et al., 1984a). Francis et al. (1978) have introduced a number of derivatizing reagents with a flophemesyl (pentafluorophenyldimethylsilyl) group, capable of forming derivatives of alcohols, phenols, carboxylic acids and amines. These latter workers report good chromatographic properties, thermal stability and high ECD sensitivity of the derivatives, but unfortunately they are prone to hydrolysis. The report indicates that the flophemesyl group ($C_8H_6F_5Si$, mol. wt. 225) increases the retention time appreciably. This makes it suitable for attachment to small molecules but unsuitable for molecules with several hydroxyl groups. The authors claim ECD sensitivity of picogram to femtogram range. These reagents warrant further investigation for use in the analysis of bioactive amines in biological tissue.

In the above section all the derivatives reported were prepared under anhydrous conditions except for some specific examples. The examples illustrating the use of halogenated acylating reagents in aqueous environment are discussed in more detail in Section C.4 (Acylation of amines in aqueous medium).

C.4 Acylation of amines in aqueous medium

There are a number of reasons for acylating amines in an aqueous medium. Amines are polar in nature and will therefore favour a polar medium such as water. Hence, the extraction of polar amines (especially those with phenol or alcohol hydroxyl groups) into a nonpolar organic solvent will not be achieved efficiently. The extraction efficiency would improve significantly if polar groups could be converted into nonpolar lipophilic groups directly in an aqueous medium. The trace amines PEA and T are present in brain in very small quantities (low ng levels). Efficient extraction of the trace amines is very important and acylation in aqueous medium will favour their partitioning into an organic phase. Polar groups generally adhere to the GLC column solid support, resulting in the "tailing" of eluting components. Acylation will improve the chromatographic performance of the amine by reducing its polarity. Structurally similar amines may co-chromatograph and acylation may be employed to isolate and separate these amines. It is also possible to acylate the amines in an aqueous medium with certain halogen-containing acylating reagents and so introduce functional groups sensitive to ECD. Thus aqueous acylation will reduce the polarity of amines, thereby improving extraction efficiency and chromatographic performance. It may facilitate separation of structurally similar amines and may also introduce functional groups highly sensitive to ECD-GLC detection.

Chattaway, in 1931, reported early work on the acylation of amines in aqueous solution. He employed acetic anhydride (AA) and observed that phenolic as well as primary aliphatic amines could be efficiently acetylated directly in aqueous solution under slightly basic conditions. Similar findings were also reported by Hagopian _et al._ (1961). Subsequent work by Brooks and Horning (1964), Laverty and Sharman (1965), and Roder and Merzhauser (1974) reported acetylation of phenolic hydroxyl groups in aqueous medium, but found that alcoholic hydroxyl groups did not acetylate under these conditions. The basis of this type of aqueous acylation is that the phenolic and amino groups are sufficiently reactive to be acetylated in aqueous solution more rapidly than the hydrolysis of the acetylating reagent. Many other acylating reagents have been employed. Hiemke _et al._ (1978) prepared propionyl, n-butyryl, isobutyryl, and pivaloylamines in aqueous solution. Propionylated amines were reported to give favourable gas chromatographic properties. The authors investigated 23 propionylated catecholamines, phenylethylamines, and indolealkylamines prepared in aqueous solution. Although ease of preparation and stability of the derivatives are advantageous, the limits of detection of their method was not reported. One way to enhance sensitivity of the acetylated or propionylated amine is to introduce a second functional group sensitive to electron capture. This is usually done by reacting the acetylated or propionylated amine with halogen-containing acylating reagents under anhydrous conditions.

The reagents TFAA, PFPA, HFBA, and PFBC have been employed in this way for the analysis of biogenic amines of the CNS. Warsh et al. (1977) reported an assay method for T employing aqueous acetylation followed by trifluoroacylation. Tryptamine was first acetylated at basic pH using acetic anhydride dissolved in diethyl ether. The organic phase containing N-acetyltryptamine was then evaporated to dryness under N_2, and the residue was reacted with a mixture of TFAA in ethyl acetate (5:1) for 1 h at room temperature. Analysis was performed with GLC-MS in the single-ion monitoring (SIM) mode. The method was applicable to urine as well as brain tissue. Martin and Baker (1977) developed an ECD-GLC method for the analysis of PEA in rat brain tissue employing aqueous acetylation. The method utilized ion-exchange resin (Amberlyst 15) for the initial isolation of the amine. The amine was eluted from the resin with methanolic-HCl and the eluate evaporated to dryness in a rotary evaporator under vacuum. To the residue, water was added together with a small quantity of sodium bicarbonate followed by 1/10 volume of acetic anhydride. After effervescence had ceased and the solution was basic, the N-acetyl derivative of PEA was extracted with ethyl acetate. The ethyl acetate layer was evaporated to dryness at room temperature under a stream of nitrogen. To the dried residue PFPA and ethyl acetate in a ratio of 5:1 were added, and the reaction was allowed to proceed for 30 min at 60°. The reaction mixture was partitioned between a small volume of cyclohexane (400 µl) and a much larger volume of a saturated solution of sodium tetraborate (4 ml) to remove the excess derivatizing reagent. The cyclohexane layer, which contained the N-acetyl,N-pentafluoropropionyl-PEA, was retained for GLC use. The authors reported several advantages in employing aqueous acetylation: it functioned as an additional purification step, resulted in amine recovery of > 95% from aqueous phase (with no contamination from inorganic salts), and finally the N-acetyl-PEA was insufficiently volatile to be lost on evaporation of ethyl acetate.

Di-(2-ethylhexyl)phosphoric acid (DEHPA) is a liquid ion-pairing reagent which was employed by Temple and Gillespie (1966) for isolation of amines. It was subsequently adopted by Martin and Ansell (1973) for use in a sensitive gas chromatographic method for estimation of NA, DA, and 5-HT in rat brain. This ion-pairing compound is now used regularly for isolation of a number of amines and amino acids prior to aqueous acetylation. A sample of urine or brain homogenate is extracted with DEHPA (2.5% v/v in chloroform), back-extracted into 0.5 N HCl and acetylated with acetic anhydride after basification. The acetylated amines are extracted with ethyl acetate, which is evaporated to dryness. The residue is reacted with 75 µl PFPA in 25 µl ethyl acetate for 30 min at 60°. The reaction mixture is partitioned between 300 µl of cyclohexane and 3 ml of saturated solution of sodium tetraborate. The organic layer, which now contains N-acetyl,N-pentafluoropropionyl derivatives of amines, is used for ECD-GLC analysis. This general procedure has been modified for the analysis of T and 5-HT (Baker _et_ _al._, 1979; Calverley _et_ _al._, 1980). Prelusky (1983) employed this method and prepared acetylated PFP and TFA derivatives for the analysis of _p_-hydroxyamphetamine (_p_-OH-AM). Tranylcypromine in rat brain regions has also been successfully analysed by this method, employing either PFPA and TFAA after aqueous acetylation. The procedure had an added advantage in that interference by endogenous N-acetyl-TCP, a metabolite of TCP, was prevented with the use of DEHPA and back-extraction with HCl prior to aqueous acetylation (Calverley _et_ _al._, 1981a). The derivatizing reagent PFBC (after aqueous acetylation) has also been employed in this manner for rapid and sensitive analysis of TCP and PEA, with reaction proceeding for 60 min at 80°. The reaction mixture was dried under a stream of N_2 and 300 µl of toluene added prior to ECD-GLC analysis of N-acetyl,N-pentafluorobenzoyl derivatives of TCP and PEA. Chemical structures of these derivatives and their mass spectral fragmentation pattern are illustrated in Figures 39 and 41 (Hampson _et_ _al._, 1984a,b).

The procedures described above have been successfully applied in the analysis of non-phenolic arylalkylamines and are also applicable to phenolic amines, although with these latter amines there may be a disadvantage. The primary amino group retains an active hydrogen atom after acetylation in aqueous medium which can subsequently be replaced by a halogen-containing moiety after reaction with a suitable acylating reagent under anhydrous conditions. However, in the case of amines with phenolic hydroxyl groups, only one active hydrogen atom is available for reaction from each phenol. Hence, when acetylated the active hydrogen atom on the phenolic hydroxyl group is replaced with an acetyl group. Although this lowers the polarity of the amphoteric phenolic amine (which facilitates extraction in the nonpolar organic solvent), the phenol group is blocked from further derivatization since it no longer possesses an active hydrogen atom for exchange. The O-perfluoroacyl derivatives have been reported to be generally more sensitive to ECD than the amino derivatives (Clarke _et_ _al._, 1966). It would, therefore, be of great advantage to develop a method in which the O-acetyl groups are selectively hydrolysed and then perfluoroacylated. This would then result in enhanced sensitivity, increased volatility (and therefore shorter retention times), and also efficient separation. The procedure of Martin and Baker (1977) has been modified for successful selective hydrolysis of the O-acetate groups after extraction (Coutts et al., 1980c). The first few steps of the method were as described earlier, employing DEHPA (2.5% v/v in chloroform) for extraction of the amines, back-extraction into 0.5 N HCl followed by basification with solid sodium bicarbonate, and acetylation with acetic anhydride. The acetylated amines, both phenolic and non-phenolic, are then extracted in ethyl acetate. The ethyl acetate layer is now divided into two portions, one for direct perfluoroacylation of non-phenolic amines (T and PEA) as described earlier, and the second portion for specific hydrolysis of the O-acetyl groups prior to perfluoroacylation. This second portion of the ethyl acetate phase is shaken with 1/10 the volume of 10 N NH_4OH solution for 40 min to hydrolyse the acetylated phenolic groups. The aqueous phase is then neutralized with 6 N HCl and the retained ethyl acetate layer taken to dryness under a stream of N_2. The residue is then reacted with 75 µl TFAA and 25 µl ethyl acetate for 30 min at room temperature. The reaction mixture is partitioned between 300 µl cyclohexane and 3 ml of saturated solution of sodium tetraborate. An aliquot of the cyclohexane is used for ECD-GLC analysis. This method has been employed for measuring m- and p-TA levels simultaneously in urine (Coutts et al., 1980b), p-TA levels in brain (Baker et al., 1982c), and for the simultaneous extraction of a number of amines, including p-OA, NME, and 3-MT (Coutts et al., 1980c; LeGatt et al., 1981; Baker et al., 1981; Baker et al., 1984b; Coutts et al., 1984a). A similar procedure was also adopted by Prelusky (1983) for analysis of p-OH-AM and p-hydroxynorephedrine in rat brain. All the above mentioned procedures have in common aqueous acetylation followed by derivatization with halogenated acylating reagents in anhydrous conditions. The phenolic arylalkylamines undergo selective hydrolyses followed by perfluoroacylation to achieve the desired sensitivity and gas chromatographic performance. However, it would be of great advantage if the arylalkylamines could be acylated in aqueous medium with a halogen-containing acylating reagent. This would then shorten the analytical procedure, since the arylalkylamines would be derivatized with ECD-sensitive reagents directly in aqueous solution. They would no longer require acetylation followed by derivatization in anhydrous conditions (requiring heat in most cases). The phenolic arylalkylamines too would be derivatized with halogenated acylating reagents in aqueous medium. Thus, they would not have to undergo a lengthy hydrolysis procedure, since the sensitivity and gas chromatographic properties of the O-perfluoroacyl derivative is much better than the O-acetyl derivative. Specific acylation of the amines can function as a selective isolation and purification procedure. In addition, it can also facilitate efficient extraction of both primary and phenolic arylalkylamines from the aqueous medium to the organic phase. Thus it appears that direct aqueous acylation with a halogenated reagent would be of great advantage to the analyst, providing him with a rapid, sensitive and selective assay method. Despite these obvious advantages, little work appears to have been done using halogen-containing acylating reagents in aqueous solution for derivatization of biogenic arylalkylamines. This is probably due to the fact that halogenated acylating reagents are generally unstable in the presence of water. Acetic anhydride (AA) has been successfully employed as a reagent for aqueous acetylation of amines, so it would be logical to substitute the protons of the acetyl group of AA with one, two, or three halogens and use the resulting compounds as acylating reagents directly in aqueous solution. A number of researchers have realized the potential of performing aqueous acylation with halogen-containing acylating reagents. Argauer (1968) employed chloroacetic anhydride (CAA, chlorine substituted for one proton on the acetyl group of acetic anhydride) for aqueous chloroacetylation of 32 phenols. Coutts et al. (1980a) analyzed a number of hydroxylated amines: phenylephrine, p-TA, p-OA, p-OH-AM, ephedrine, norephedrine, morphine, codeine, and hydroxylated aniline isomers in aqueous solution with CAA and dichloroacetic anhydride (DCAA). Recently, Baker et al. (1984a) have developed a novel analytical procedure employing aqueous trichloroacetylation for a rapid and sensitive analysis of PEA and TCP in the same piece of brain tissue. Trichloroacetic anhydride (TCAA) (1/10 of the total volume) was added directly to the basified supernatant of the brain homogenate. The trichloroacetyl derivatives were extracted in ethyl acetate after the effervescence had ceased. The organic layer was dried under a stream of N_2 and to the dried residue 100 µl of toluene was added. After washing the toluene layer with water, an aliquot of the toluene layer was used for ECD-GLC analysis.

Benzoylation has also been employed in aqueous solution for the extraction of amines. Decroix et al. (1968) isolated amines from aqueous solution (3 ml) after basifying it with an equal volume of 7.5 M sodium hydroxide (NaOH) and adding 50 µl of benzoyl chloride. The benzamides were extracted with 2 x 1 ml of diisopropyl ether. A benzoyl chloride substituted with halogens would therefore be an obvious choice for acylation in aqueous medium. Cristofoli et al. (1982b) employed this principle and used pentafluorobenzoyl chloride (PFBC) in aqueous medium for the derivatization of a monofluorinated analogue of AM in brain tissue using ECD-GLC. A modification of this method has also been adopted for the analysis of AM and N-alkylated analogues in the same piece of brain tissue (Nazarali _et_ _al._, 1983).

Thus it would appear that aqueous acylation with PFBC, TCAA, and possibly with other halogen-containing reagents yet to be explored, promises to be a new and substantial advance in the analysis of the bioactive amines.

C.5 Synopsis for a systematic approach to selection of electron-capturing derivatives

From the review of the literature on derivatizing reagents employed in ECD-GLC analysis of amines, a number of factors have come to light in selecting derivative types. The important considerations are the volatility of the derivative, ease of preparation, and the detection sensitivity. These considerations are rarely independent of one another, and a compromise on the type(s) of derivative(s) employed will be dependent on the analytical priorities of the chromatographer. Fluorinated derivatives are usually preferred over chlorinated ones as they are likely to be more volatile and separation can be achieved at lower temperatures. With compounds that are difficult to volatilize, generally HFB or PFP derivatives are used to confer volatility. The trimethylsilyl (O-TMS) derivatives confer stability but are less volatile than O-HFB or O-PFP derivatives (and have poor sensitivity on ECD). However, the N-HFB or N-PFP derivatives are generally much more stable than the N-TMS derivatives. The choice of derivatives employed for moderately volatile compounds is somewhat greater. Pentafluorobenzoyl or TCA derivatives have been used for optimum ECD response. Mixed derivatives such as one containing a PFB and a TMS group can be used for lowering the temperature of analysis for compounds that may elute at temperatures close to the limit of the column. For compounds that are highly volatile, the approach taken would be to decrease their volatility so as to avoid loss during evaporation and yet confer stability and sensitivity. To do this, large electron-capturing groups such as flophemesyl, PFB, or pentafluorobenzyl are used. Aqueous acetylation is also being used more prominently and has a two-fold advantage: firstly, the extraction efficiency of trace levels of volatile amines is significantly improved, and secondly, the highly volatile compound is stabilized. Aqueous acylation with halogen-containing reagents such as PFBC or TCAA has recently been employed with considerable success and holds promise for future use. The derivatives prepared for ECD analysis can also be suitably employed with other detector systems such as FID or thermal conductivity and will impart good chromatographic properties although they may not be as sensitive to detection.

Thus it appears that choices of derivatives employed need not necessarily be empirical as it has been in the past, and that there is some indication of a more systematic approach to selection of specific derivatives. Undoubtedly, future research in the application of derivatives for chromatographic analysis by numerous scientists in various fields will lead to a more organized approach for selection of derivatives.

D. Pro-drugs and the CNS

D.1 Introduction

A pro-drug is a chemical modification of a drug to form a new compound which in vivo liberates the parent compound. The chemical modification of the parent compound is such that it alters its duration of action, its efficacy, or even causes it to have a different pharmacological action. Albert (1958, 1968) first introduced the term "pro-drug" to describe compounds which underwent biotransformation prior to exhibiting their pharmacological effects. The pro-drug approach has been very successful in the design of stable, safe and efficient drugs. Factors which have limited the full development or successful screening of novel pharmacologically active agents can be overcome with the use of a pro-drug approach (Fig. 4). A drug can be rejected due to pathological complications such as increased incidences of side effects and toxicity. Formulation problems or chemical instability are other limiting factors. Important considerations such as unpleasant odour or taste and pain at injection site are common physiological and psychological limitations. Perhaps a significant factor is the potential a drug has to make economic gains for its manufacturer. A large company may not be interested in marketing an unpatented drug with physico-chemical properties limiting its usefulness. However, if a chemical modification could be successfully employed which will remove the barrier to the drug's use, then the product may have an economic potential (Stella, 1975).

Recent resurgence in pharmacokinetics (a study of the time course of absorption, distribution, metabolism and excretion of drugs) has increased awareness among the scientific community that a drug can only

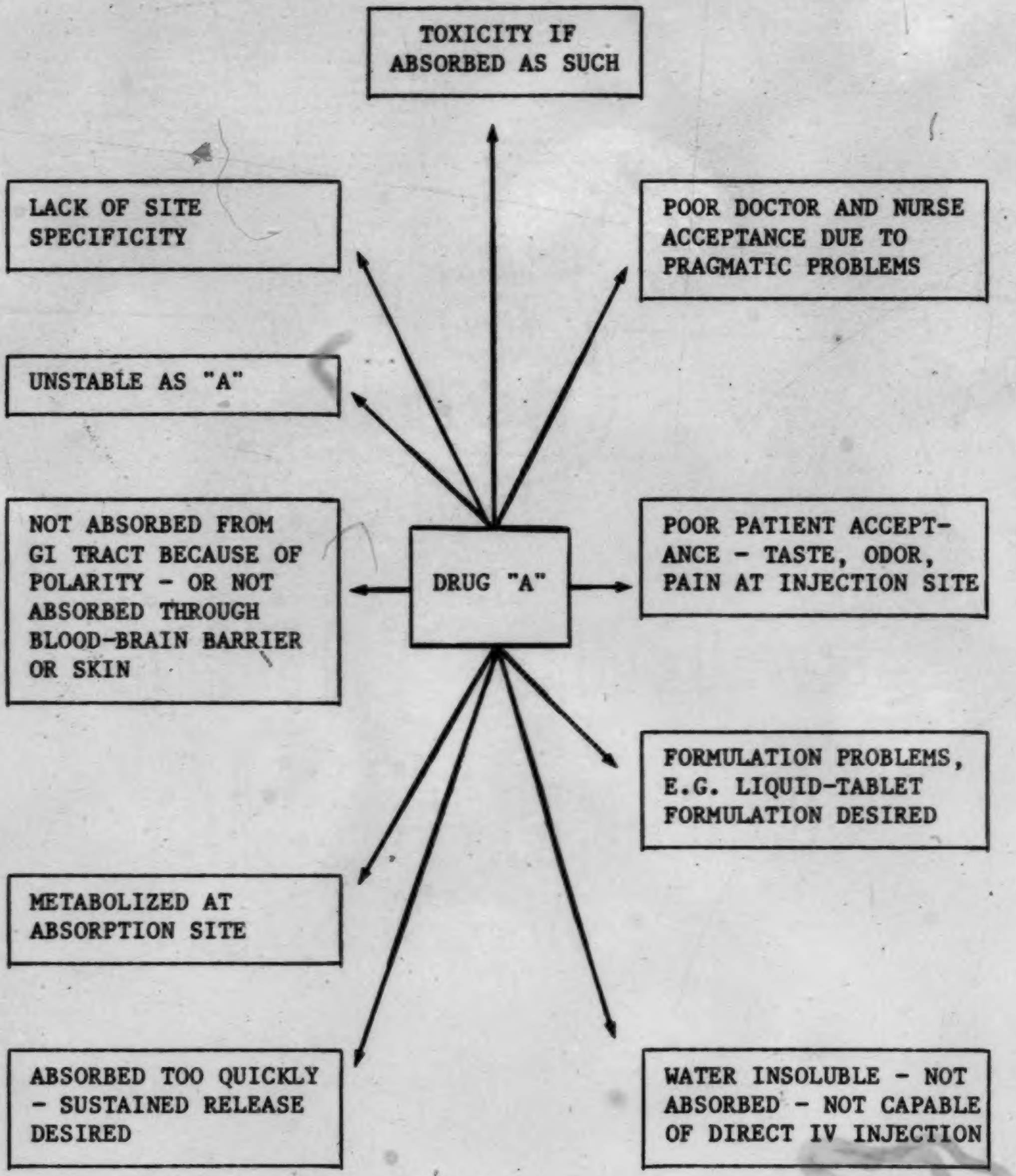

TOXICITY IF ABSORBED AS SUCH
LACK OF SITE SPECIFICITY
POOR DOCTOR AND NURSE ACCEPTANCE DUE TO PRAGMATIC PROBLEMS
UNSTABLE AS "A"
NOT ABSORBED FROM GI TRACT BECAUSE OF POLARITY - OR NOT ABSORBED THROUGH BLOOD-BRAIN BARRIER OR SKIN
DRUG "A"
POOR PATIENT ACCEPTANCE - TASTE, ODOR, PAIN AT INJECTION SITE
FORMULATION PROBLEMS, E.G. LIQUID-TABLET FORMULATION DESIRED
METABOLIZED AT ABSORPTION SITE
ABSORBED TOO QUICKLY - SUSTAINED RELEASE DESIRED
WATER INSOLUBLE - NOT ABSORBED - NOT CAPABLE OF DIRECT IV INJECTION

Fig. 4. Factors which limit successful screening and/or full development of novel drugs (adapted from Stella, 1975).

exert a desired pharmacological effect if it reaches its site of action. A number of limitations have to be overcome before a drug can reach the target organ where it will interact with the receptor. The drug has to reach an effective and desired concentration at the target organ. Hence a time-concentration profile of a drug and its metabolites in the target organ and other tissues has to be performed to assess the drug's effectiveness. A change in the physico-chemical properties of a drug because of its conversion to a pro-drug can affect the time profile of the parent drug in the various compartments. This alteration in time-concentration profile of the parent drug can be employed to advantage. A number of examples below illustrate the pro-drug approach, taken to solve specific problems.

D.2 β-Phenylethylamines

Transport of low molecular weight amines across the blood-brain barrier (BBB) appears to depend on two factors, lipophilicity and biotransformation. The lipid solubility and the concentration of the unionized moiety of the amine in the blood determine the rate of transport across the BBB. This concept has been useful in correlating the action of centrally active drugs and is termed the "lipophilicity theory" (Mayer et al., 1959; Brodie et al., 1960; Hansch et al., 1968; Dewhurst, 1970; Oldendorf and Dewhurst, 1978). However, in the case of amines, the metabolizing enzyme barrier as well as the lipoidal membrane barrier are important factors for transport across the BBB. The presence of MAO and other deactivating enzymes will metabolize phenylethylamines before their entry into the brain (Bertler et al., 1966). The amines that are capable of entry into the brain may be rapidly metabolized by enzymes present in brain before they can reach their proper site of activity.

To facilitate transport of these amines across the BBB and to ensure that an adequate concentration reaches the site of activity, attempts to chemically modify these amines have been made with some success. Verbiscar and Abood (1970) synthesized a number of carbamate esters of the physiologically active amines PEA, AM, ephedrine, and p-OH-AM. In their study they found that the anorexiant property of the carbamates occurs with less central stimulation than with AM and that the carbamates provide delayed onset and greater duration of action. The principle for employing this approach is that carbamoylated amines do not ionize readily and are therefore more soluble in lipoidal membranes. Success with the carbamate pro-drug of the amines requires that the drug be hydrolyzed to a carbamic acid and an alcohol moiety after entry into the brain. Carbamic acids are unstable at physiological pH, forming the parent amine and carbon dioxide (CO_2) (Olsen et al., 1952). This decarboxylation of the carbamic acid can be compared to the formation of CO_2 by endogenous amino acids such as 5-hydroxytryptophan or phenylalanine which decarboxylate in brain to form the biogenic amines 5-HT and PEA, respectively. The alcohol leaving group may be varied to alter the formation characteristics of the parent amine in the brain. Ethanol has been favoured for use as a leaving group because of its low toxicity (Bjurulf et al., 1967; Kupchan and Isenberg, 1967; Baker et al., 1984c). Bjurulf et al. (1967) conducted a comprehensive clinical study (employing double-blind and cross-over techniques) on the N-ethoxycarbonyl analogue of chlorphentermine (Oberex), a pro-drug of the anorexiant chlorphentermine. In their study of the pro-drug they conclude that "one advantage of the preparation is its relatively prolonged effect, which makes one dose in the morning apparently sufficient." The N-ethoxycarbonyl analogue of normeperidine has been tested for its analgesic activity and found to have high potency with no toxicity or physical dependence capacity at doses ranging from 1.0 to 130.0 mg/kg (in monkey) (Kupchan and Isenberg, 1967). A recent study with the N-ethoxycarbonyl analogues of PEA, AM and TCP indicates substantial conversion of N-ethoxycarbonyl analogue to the parent amine (Baker et al., 1984c; Results section of this thesis). Both the analogue and parent amine levels were detected in brain and the amounts of each present in brain were quantitated using a novel sensitive ECD-GLC method. The study showed that the N-ethoxycarbonyl analogue of TCP was inactive against MAO enzymes when tested in vitro; however, when the analogue was administered to rats, the brain MAO enzymes were found to be inhibited in vivo. This suggested that the MAO-inhibiting effect observed in vivo was due not to the pro-drug, but to the TCP formed from the pro-drug. Tranylcypromine is a clinically used antidepressant of the MAO-inhibitor type. We also found that administration of the N-ethoxycarbonyl analogue of PEA elevated the brain levels of PEA, and concluded that this may be a method for selectively increasing brain concentrations of PEA without affecting levels of other biogenic amines.

D.3 Catecholamines

In Parkinson's disease, deficiency in brain DA, resulting from the degeneration of the substantia nigra (Hornykiewicz, 1966, 1984; Barbeau, 1967), has led to several attempts to raise the brain levels of DA in these patients. At physiological pH, DA is ionized and highly polar and is incapable of crossing the BBB. However, L-Dopa (L-3,4-dihydroxyphenylalanine), a precursor or a pro-drug of DA which crosses the BBB via the amino acid active transport system, is successfully used in the treatment of Parkinson's disease (Barbeau, 1969; Calne et al., 1969; Mawdsley, 1970; Peaston and Bianchine, 1970). The enzyme Dopa-decarboxylase converts L-Dopa to DA in the CNS (Fig. 5). Unfortunately, only about 20% of an orally administered dose of L-Dopa is available to cross the BBB, since most of it gets decarboxylated in the periphery (Rivera-Calimlim et al., 1970; Stella, 1975; Martindale, 1982c). This "first-pass" effect varies from individual to individual and depends on the patient's age, diet, and severity of illness. Concurrent administration of an inhibitor of decarboxylase at the peripheral sites, such as carbidopa (α-methyldopa), which does not cross the BBB, makes available a higher percentage of L-Dopa, thereby enabling the dose of L-Dopa to be reduced, and this may diminish some side-effects.

However, other attempts to elevate brain DA levels have been to synthesize esters of L-Dopa (Lai and Mason, 1973) or the methyl ester of tyrosine (Anden et al., 1966) to prevent peripheral decarboxylation. Other analogues such as the 3,4-diacetyldopamine (DADA) or the 3,4-di-(trimethylsilyl)dopamine, illustrated in Figure 6, have been suggested as possible pro-drugs of DA (Pinder, 1970). It is suggested that the presence of the diacetyl groups will protect the hydroxyl groups on the ring from being conjugated and would also facilitate passage through the BBB due to increased lipophilicity of the molecule. However, Borgman et al. (1973) could not find evidence of increased DA activity in mice treated with DADA.

Prolonged formation of noradrenaline (NA) in mouse brain was

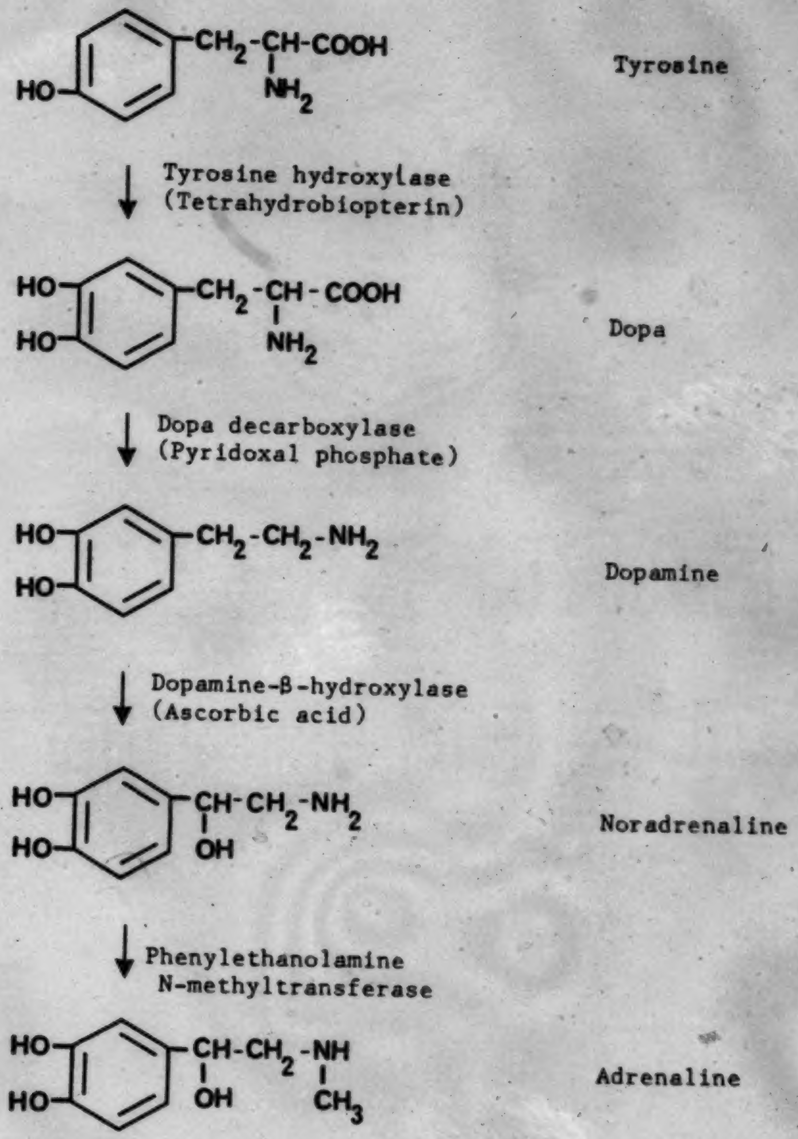
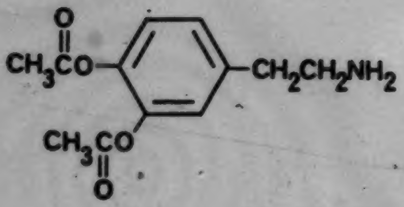
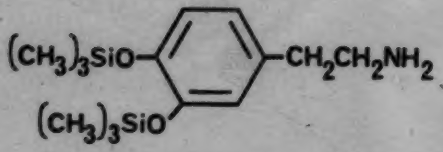

Tyrosine
Tyrosine hydroxylase
(Tetrahydrobiopterin)
Dopa
Dopa decarboxylase
(Pyridoxal phosphate)
Dopamine
Dopamine-β-hydroxylase
(Ascorbic acid)
Noradrenaline
Phenylethanolamine
N-methyltransferase
Adrenaline

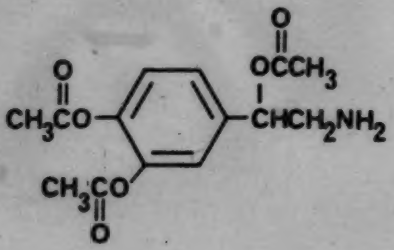
Fig. 5. Synthesis of catecholamines (adapted from Lader, 1980a). Cofactors are indicated in parentheses.



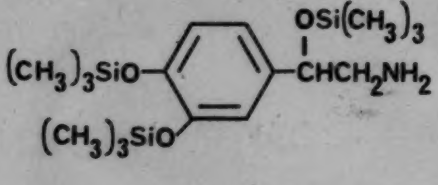
3,4-diacetyldopamine (DADA)



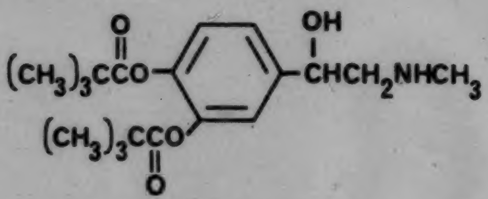
3,4-di(trimethylsilyl)dopamine



3,4,β-triacetylnoradrenaline



3,4,β-tri(trimethylsilyl)noradrenaline



Dipivalyladrenaline

Fig. 6. Pro-drugs of the catecholamines dopamine, noradrenaline and adrenaline.

reported by Daly et al. (1966) and Creveling et al. (1969) after administration of 3,4,β-triacetyl and 3,4,β-tri(trimethylsilyl) analogues of NA. Adrenaline (A, epinephrine) has been used in the treatment of glaucoma; however, because of its side effects (ocular and systemic) and instability in aqueous solution, a pro-drug to counter these problems was synthesized (McClure, 1975). Acylation of the phenolic hydroxy groups to give the dipivalyl analogue (Fig. 6) was found to decrease side effects, increase stability, as well as increase therapeutic effectiveness of A by a factor of approximately 100. The dipivalyl pro-drug of A had significantly increased lipophilicity compared to A, and as absorption into the cornea of the eye involves transport through a lipoidal membrane, the high lipophilicity of the pro-drug may account for its superior therapeutic effectiveness.

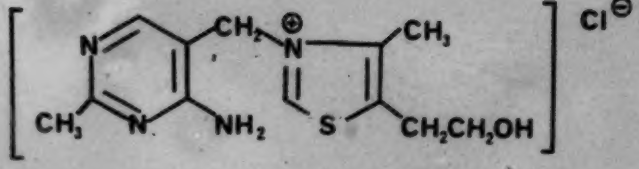
D.4 Thiamine

Thiamine (vitamin B_1) is a water-soluble compound containing a pyrimidine and a thiazole nucleus with a quaternary nitrogen and is illustrated in Figure 7. Thiamine is poorly absorbed both in the CNS (Cohen et al., 1962) and the gastrointestinal (GI) tract (Thomson et al., 1968, 1970; Rindi and Ventura, 1972) and is absorbed into the CNS and from the GI tract by an active transport mechanism. The active transport of thiamine requires energy and is therefore saturable and/or easily inhibited. Chronic alcohol consumption is accompanied by low thiamine intake and in addition may inhibit oral absorption of thiamine; this has been implicated in Wernicke's encephalopathy (Thomson et al., 1971). Certain characteristic signs of this condition are ophthalmoplegia, nystagmus, and ataxia. However, Wernicke's syndrome is often accompanied by signs of Korsakoff's psychosis, characterized by the patient's inability to acquire new information, impaired memory, and confabulation (Greengard, 1975). Recently, Butterworth (1983) has shown that severe thiamine depletion not only causes encephalopathy of the mammalian CNS but also results in regio-selective changes in neurotransmitter function. In the cerebellum of thiamine-deprived rats, the concentrations of glutamate and γ-aminobutyric acid (GABA) were shown to be selectively reduced.

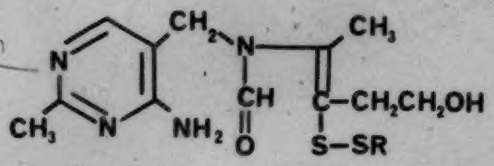
Subacute Necrotizing Encephalomyelopathy (SNE, Leigh's Disease) is a genetic disease in children with brain lesions very similar to those in Wernicke's encephalopathy. Inhibited CNS absorption of thiamine has been implicated in this disease (Montpetit et al., 1971; Pincus, 1972).

In Japan, the practice of rice polishing led to some thiamine deficiency. Thiamine could not be used as a food additive as it was found to be too water soluble and easily washed from rice (Stella, 1975). Thiamine is also chemically unstable (Windheuser and Higuchi, 1962) and poorly absorbed. Hence a number of thiamine derivatives were synthesized (Matsukawa et al., 1962) as possible food additives and some were later tested as lipid soluble pro-drugs of thiamine for preferential GI or CNS absorption.

The derivatives of thiamine no longer possess a quaternary nitrogen and are therefore able to penetrate the GI tract by passive absorption. The disulfide pro-drugs thiamine propyldisulfide (TPD) and thiamine tetrafurfuryldisulfide (TTFD), illustrated in Figure 7, are metabolized to thiamine in the presence of glutathione and glutathione reductase (Nogami et al., 1969a,b,c, 1973). Thioesterases and esterases metabolise the derivatives O,S-diacetylthiamine (DAT) and O,S-diethoxycarbonyl-

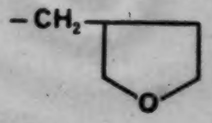


Thiamine

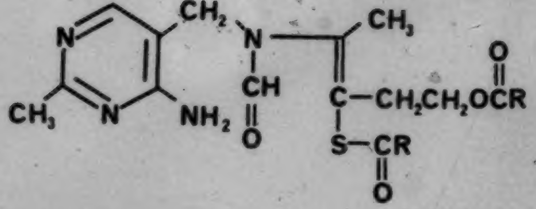


When R is $-C_3H_7$; Thiamine propyldisulfide (TPD)

R is



; Thiamine tetrafurfuryldisulfide (TTFD)



When R is $-CH_3$; O,S-diacetylthiamine (DAT)

R is $-OC_2H_5$; O,S-diethoxycarbonylthiamine (DECT)

Fig. 7. Thiamine and pro-drugs of thiamine.

thiamine (DECT) (Kawasaki, 1963).

Thomson et al. (1971) conducted a clinical study with the TPD analogue in patients with Wernicke's encephalopathy as well as in alcoholic patients with symptoms similar to, but not necessarily suffering from, Wernicke's disease. Figure 8 is a time-concentration profile of thiamine in blood of normal subjects and of malnourished alcoholic patients with fatty liver after administration of 50 mg dose of thiamine HCl compared to the same dose of TPD. The results indicate that the derivative TPD forms substantial amounts of thiamine in blood (compared to levels reached after thiamine alone) in both the alcoholic patient and normal subject. Their studies have also indicated that TPD increases and in some cases normalizes the erythrocyte transketolase activity (ETA) in alcoholic, thiamine-deficient patients, whereas thiamine alone had a small effect on ETA. Of a group of 6 patients with Wernicke's encephalopathy, 5 showed complete remission of ocular palsy just six hours after administration of TPD and the sixth patient showed an improved condition. A comparison of clinical and laboratory response to a 50 mg dose of thiamine HCl followed by the same dose of TPD in thiamine-deficient alcoholics is illustrated in Figure 9. It can be observed that with the administration of TPD the thiamine levels are elevated, resulting in decreased lateral rectus palsy. Thiamine propyldisulfide has also been used in Leigh's disease with some success (Pincus, 1972). Preliminary clinical investigation with the O,S-diacetylthiamine (DAT) in Leigh's disease has also claimed beneficial effects (Stella, 1975).

D.5 Neuroleptics

The long-acting injectable (depot) neuroleptics have made a signi-

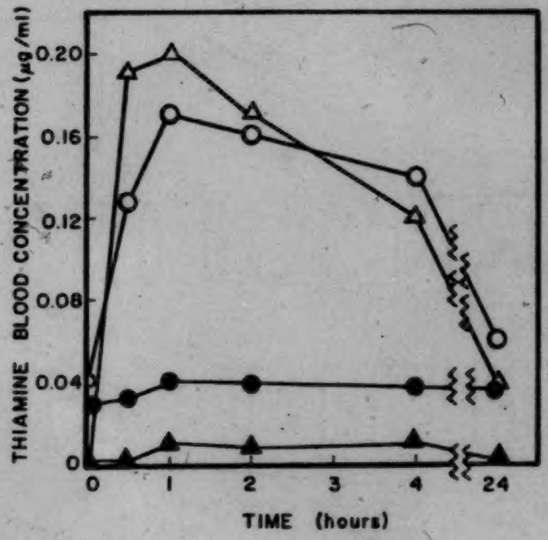


Fig. 8. Thiamine blood levels in malnourished alcoholic patients with fatty livers (Δ) and in normal subjects (O) after ingestion of 50 mg of thiamine propyldisulfide (TPD) (open symbols) or 50 mg of thiamine hydrochloride (closed symbols) (adapted from Thomson et al., 1971).

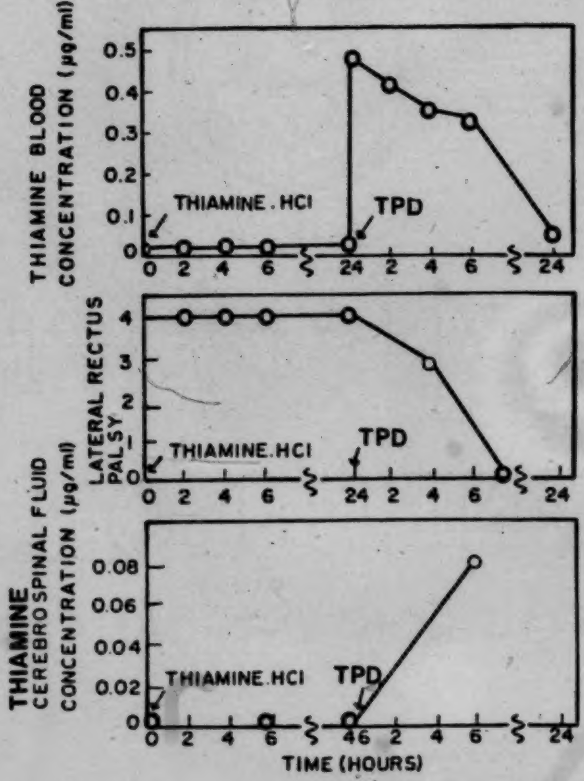
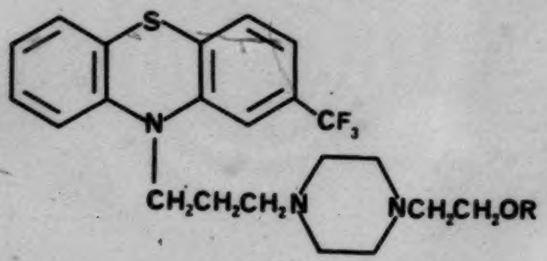


Fig. 9. Comparison of the response of clinical and laboratory abnormalities to 50 mg of thiamine hydrochloride followed by thiamine propyldisulfide (TPD) in thiamine deficient alcoholics (adapted from Thomson _et al._, 1971).

ficant impact on the treatment of chronic ambulatory schizophrenic patients. The neuroleptics are administered intramuscularly (i.m.) every two to four weeks (Lader, 1980b). Fluphenazine enanthate and fluphenazine decanoate are fluphenazine esters in sesame oil vehicle, and when given by i.m. injection have a prolonged activity for up to two to four weeks (Ebert and Hess, 1965; Dreyfus et al., 1971; Knights et al., 1979; Martindale, 1982d). Other depot neuroleptic drugs which are esters in an oil vehicle are flupenthixol decanoate, pipothiazine palmitate, and pipothiazine undecenoate, illustrated in Figure 10 (Jorgensen et al., 1971; Villeneuve et al., 1972; Julou et al., 1973; Nymark et al., 1973; Villeneuve and Fontaine, 1980).

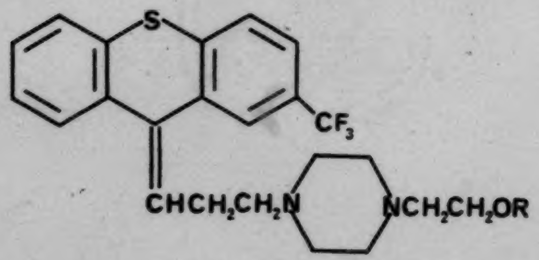
The long-chain fatty acid formulation makes the neuroleptic highly lipophilic and when given by i.m. it dissolves in the fatty tissue of the muscle, maintaining a sustained release of the neuroleptic. Figure 11 is a comparison of the effect of an oral daily dose of flupenthixol hydrochloride (5 mg/kg) to flupenthixol decanoate (10 mg/kg, single i.m. injection) on the inhibition of a conditioned avoidance response (Nymark et al., 1973). It can be observed that the ester (flupenthixol decanoate) resulted in a sustained response, compared to the flupenthixol hydrochloride, which showed "peak" and "valley" responses. Metabolic studies with the esters of fluphenazine (Dreyfus et al., 1971), esters of flupenthixol (Jorgensen et al., 1971), and esters of pipothiazine (Villeneuve et al., 1972) have shown that the activity of the esters was related to the formation of the parent compound. The rationale for using these ester pro-drugs of neuroleptics is to avoid the "first-pass" metabolism in the liver; the drug is released directly into the systemic circulation from its "depot" in the muscle. This leads to



When R is -H ; Fluphenazine

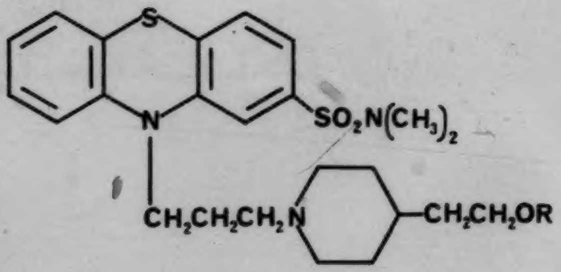
R is $-COC_6H_{13}$; Fluphenazine enanthate

R is $-COC_9H_{19}$; Fluphenazine decanoate



When R is -H ; Flupenthixol

R is $-COC_9H_{19}$; Flupenthixol decanoate



When R is -H ; Pipothiazine

R is $-COC_{15}H_{31}$; Pipothiazine palmitate

R is $-COC_{10}H_{21}$; Pipothiazine undecenoate

Fig. 10. Chemical structures of the neuroleptics fluphenazine, flupenthixol, pipothiazines and their ester pro-drugs.

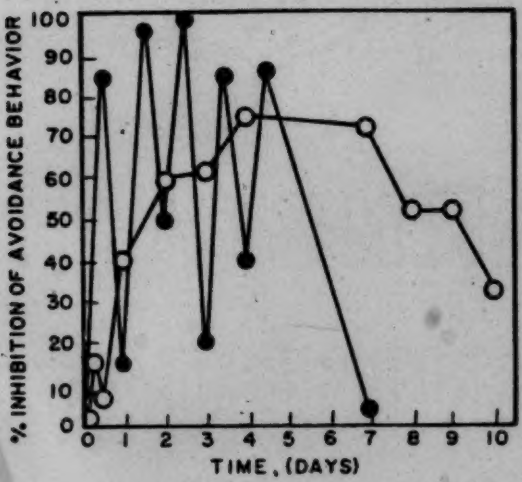


Fig. 11. Days after drug administration--single i.m. dose of flupenthixol decanoate in oil (○) or flupenthixol oral daily (●) (adapted from Nymark *et al.*, 1973).

a prolonged sustained release of the neuroleptic. Another important benefit from these "depot" neuroleptics has been an improvement in patient compliance (Lader, 1980b)--the psychiatrist who prescribes the neuroleptic will know that the injectable neuroleptic has been administered. This is important because the severely psychotic patient may fail to adhere to the prescribed regimen of oral medication.

D.6 Conclusion

From the examples discussed in this section it can be concluded that the concept of pro-drugs has produced a number of useful and potentially useful drugs for use in the treatment of CNS diseases. Many pharmaceutical companies are engaging in pro-drug research as regulatory agencies in many countries become more stringent, requiring increased information with every new drug application. The pro-drug approach can help the manufacturer gain further patent coverage on older products or improve the safety of delivery of new products. Thus millions of dollars are at stake and information regarding novel analogues would be jealously guarded by the manufacturer.

Advantages of employing pro-drugs or sustained release products (Stella, 1975) are:

1. reduction in the frequency of doses to be administered;
2. the "peak" and "valley" effects seen with conventional preparations are eliminated;
3. the desired pharmacological/therapeutic effect is often seen with a lowered total concentration of drug;
4. the problem of nighttime administration of drugs is eliminated;
5. patient compliance will improve by decreasing the number of times a

patient must remember to take his/her medication;

6. the incidence of peak blood levels rising above the toxic blood levels is reduced;
7. incidence of G.I. side effects is reduced.

These advantages and other implications discussed earlier make the pro-drug approach an exciting field for continued study.

Numerous problems associated with conventional drug treatments of CNS diseases may be overcome by the pro-drug approach. A multidisciplinary team of researchers—such as medicinal chemists with their knowledge of synthesis, structure-activity relationships, and metabolism; neurochemists with their knowledge of brain biochemistry and neurotoxicity; pharmacists with their knowledge of formulations and pharmacokinetics; and psychiatrists with their knowledge of brain function and expertise in diagnosis—can co-operate in the search for novel pro-drugs to optimize drug delivery in the treatment of CNS diseases while minimizing toxicity and unfavourable side effects of the drug.

II. MATERIALS AND METHODS

A. Chemicals and Derivatizing Reagents

A list of the sources of all chemicals, drug compounds and bioactive amines is shown in Table II, and sources of all derivatizing reagents in Table III. A Corning AG-3 or Corning Mega-Pure (3 Litre Automatic) still was used to collect double-distilled water for use in analysis. The derivatizing reagents PFBC and PFPA were stored at 0°C. Trichloroacetic anhydride was kept frozen at -10°C and thawed prior to use, and AA was stored in a fumehood at room temperature. All derivatives were prepared in a fume hood.

B. Instrumentation and Apparatus

B.1 Gas-liquid chromatography (GLC)

Gas-liquid chromatography was performed on two column types, capillary or packed, attached to an electron-capture detector with a radioactive source of 15 mCi ^{63}Ni. The two capillary GLC instruments were:

1. Hewlett Packard (HP) 5880A, equipped with a 10 m long WCOT OV-101 fused silica column (0.8 mm o.d. and 0.25 mm i.d.), coupled to an HP 5880A integrator (level 4).
2. Hewlett Packard 5880A equipped with a 12 m long WCOT OV-1 fused silica column (0.8 mm o.d. and 0.50 mm i.d.) coupled to an HP 5880A integrator (level 2).

The operating conditions for both instruments were the same. Helium (2 ml/min) was used as the carrier gas, and 10% methane in argon at a flow

Table II. A list of chemicals, drug compounds and bioactive arylalkylamines employed in this study.

Chemical, Drug Compound or Bioactive Amine	Source or Manufacturer
Acetonitrile	Fisher Scientific Ltd.
Ammonium hydroxide	Fisher Scientific Ltd.
(±)Amphetamine HCl	Smith, Kline and French Laboratories
2-(4'-tert-Butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole (Butyl-PBD)	Sigma Chemical Co.
Chloroform, reagent grade	Caledon Laboratories Ltd.
p-Chlorophenylethylamine[1] HCl	Sigma, and Drs. T. W. Hall and R. G. Micetich
(±)N-(3-Chloropropyl)amphetamine HCl	Synthesis by Drs. F. M. Pasutto and G. R. Jones
(±)N-(2-Cyanoethyl)amphetamine HCL	Synthesis by Drs. F. M. Pasutto and G. R. Jones
N-(2-Cyanoethyl)phenylethylamine HCl	Synthesis by Drs. T. W. Hall and R. G. Micetich
(±)N-(2-Cyanoethyl)tranylcypromine oxalate	Synthesis by Drs. T. W. Hall and R. G. Micetich
N-(2-Cyanoethyl)tryptamine oxalate	Synthesis by Drs. T. W. Hall and R. G. Micetich
Cyclohexane (glass distilled)	Caledon Laboratories Ltd.
2,4-Dichlorophenoxypropylamine HCl (Ref. no. GS 26716)	May and Baker Ltd.
Di-(2-ethylhexyl)phosphate (DEHPA)	Sigma Chemical Co.
(+)N-(Ethoxycarbonyl)amphetamine (free base)	Synthesis by Dr. T. J. Danielson
N-(Ethoxycarbonyl)phenylethylamine (free base)	Synthesis by Dr. T. J. Danielson
(±)N-(Ethoxycarbonyl)tranylcypromine (free base)	Synthesis by Dr. T. J. Danielson

Table II (cont'd).

Chemical, Drug Compound or Bioactive Amine	Source or Manufacturer
Ethyl acetate (glass distilled)	Caledon Laboratories Ltd.
Ethylenediaminetetraacetic acid (EDTA)	J. T. Baker Chemical Co.
Hydrochloric acid, 37-38%	Fisher Scientific Ltd.
5-Hydroxytryptamine creatinine sulfate	Sigma Chemical Co.
Hydroxytryptamine binoxalate 5-[2-^{14}C]	New England Nuclear
(±)para-Hydroxyamphetamine	Smith, Kline and French Laboratories
Perchloric acid, 60%	Fisher Scientific Co.
β-Phenylethylamine HCl	Sigma Chemical Co.
Phenylethylamine HCl 2-[ethyl-1-^{14}C]	New England Nuclear
Potassium bicarbonate	BDH Chemicals
Potassium carbonate, anhydrous	Fisher Scientific Co.
(±)N-n-propylamphetamine HCl	Synthesis by Dr. F. M. Pasutto
Sodium bicarbonate	Fisher Scientific Co.
Sodium carbonate, anhydrous	J. T. Baker Chemical Co.
Toluene (glass distilled)	Caledon Laboratories Ltd.
(±)Trans-2-phenylcyclopropylamine HCl (Tranylcypromine)	Sigma Chemical Co.
Triton X-100	Terochem
Tryptamine HCl	Sigma Chemical Co.

[1] The p-chlorophenylethylamine was obtained from Sigma Chemical Co. as a free base, which was subsequently converted to the hydrochloride salt by Drs. T. W. Hall and R. G. Micetich (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta).

Table III. List of derivatizing reagents.

Derivatizing Reagents	Source
Acetic anhydride (AA)	Caledon Laboratories Ltd.
Pentafluorobenzoyl chloride (PFBC)	Aldrich Chemical Co.
Pentafluoropropionic anhydride (PFPA)	Pierce Chemical Co.
Trichloroacetic anhydride (TCAA)	Pfaltz and Bauer Inc.

rate of 36 ml/min was used as makeup gas at the detector. The injection port temperature was 270° and the detector temperature was 300°. The column temperature was programmed from 80 to 270° or 105 to 270° at a program rate of 20°/min or 25°/min.

For packed column analysis, the GLC employed was an HP 5830A. This instrument was equipped with a 2 m long glass column (4 mm i.d.) packed with 3% OV-17 on Gas-Chrom Q (100-120 mesh). The GLC was coupled to an HP 18850A integrator. Methane in argon (10/90) was used as the carrier gas at a flow rate of 40 ml/min. The temperature at the injection port was 270° and the detector temperature was 300°. The column temperature was maintained at 200° for 5 min and programmed to increase to 270° at a rate of 20°/min. All measurements of drug and amine concentrations were based on ratio of peak heights of the compounds of interest to the peak height of an internal standard of fixed concentration. These values were compared to standard curves prepared by adding known varying amounts of authentic standards and a fixed concentration of internal standard to series of tubes and carrying them in parallel through the assay procedure. A standard curve was prepared for each assay run.

B.2 Mass spectrometry

Mass spectra were obtained using combined GLC-MS. The quadrupole mass spectrometer was a Hewlett Packard (HP) model 5985A with dual EI/CI sources and an HP model 7920 data system. The GLC-MS system was also comprised of an HP model 5840A GLC, HP 2648A graphics terminal, HP 9876A printer, HP 7920 disc drive (software), and HP 21MX series E computer (hardware). Operating conditions were as follows: ion source temperature, 200°; interface temperature, 275°; column pressure, 10 p.s.i.;

accelerating voltage, 2200 eV; ionization voltage, 70 eV; scan speed, 100 amu/sec; and dwell time, 200 msec.

B.3 Liquid scintillation spectrometry

A Beckman model LS 7500 (microprocessor-controlled) liquid scintillation spectrometer coupled to a Datamex Model 43 printer was used in counting radioactivity in all *in vivo* and *in vitro* MAO inhibition studies. The LS 7500 is a soft-beta counting spectrometer with 300-sample capacity, equipped with Automatic Quench Compensation (AQC), H-Number quench monitor, keyboard operation, program editing feature and 10-Library counting programs in an unalterable memory.

B.4 Glassware

B.4.1 Procedure for cleaning glassware

All glass tubes were washed with tapwater and biodegradable Sparkleen (Fisher Scientific Co.). The tubes were placed in an ultrasonic cleaner (Mettler Electronics) containing a solution of Decon 75 concentrate (BDH Chemicals), 20 ml to 1 litre, and ultrasonicated for 1 h. The glass tubes were then stacked in stainless steel wire mesh baskets. The tubes were rinsed with distilled water and then rewashed in a washing machine (Miele Electronic G715) in wash-rinse mode with distilled water. Finally, the glass tubes were air-dried in a mechanical convection oven (Model 28, Precision Scientific Group). This procedure was found to be efficient in removing all adsorbed organic contaminants from glass surfaces.

B.5 Weighing balances

All sample weighing for preparation of standard solutions or weighing of brain tissue was performed with either Sartorius 2003 MP1 or Mettler AE 160 weighing balances.

B.6 Potter-Elvejhem homogenizer

Homogenization of brain tissue in perchloric acid was done using a TRI-R Model S63C variable speed laboratory motor with a Teflon® glass pestle and a glass grinding tube (clearance: 0.1-0.15 mm). The motor has a maximum speed of 12,000 r.p.m. with 10 speed levels. A speed level of 7 was used for homogenizing the brain samples.

B.7 Centrifugation

B.7.1 Bench centrifuge

This was a Sorvall® GLC-2B or Sorvall® GLC-1 General Laboratory Centrifuge (DuPont Instruments).

B.7.2 High-speed centrifuge

Centrifugation of all brain homogenate samples were performed in a Damon-IEC Model B-20 (heavy duty) refrigerated high-speed centrifuge. The operating conditions were as follows: centrifugation at 12,000 x g; time, 15 min; and temperature, 0-4°.

B.7.3 Microcentrifuge

Microcentrifugation was performed with either the MSE Micro-Centaur or the Beckman Microfuge B.

B.8 Shaker-mixer

The shaker-mixer employed was an Evapo-Mix (Buchler Instruments) at vortex speed 8. This instrument was capable of performing efficient mixing of ten sample tubes simultaneously.

B.9 Block heater

A Reacti-Therm® Heating Module (Pierce Chemical Co.) or Multi-Blok Heater® No. 2090 (Lab-Line Instruments), both with adjustable temperature control, were employed in analyses where heat was required for derivatization.

C. Animals

Male Sprague-Dawley rats (220-250 g) obtained from Bio-Science Animal Services, Ellerslie, Alberta, were used for all studies. The animals were housed in plastic cages on cedar chip bedding, in a temperature-controlled room (21°). The rats were subjected to controlled conditions of 12-hour on/12-hour off lighting schedule. Food and water were provided *ad libitum*. The Lab-Blox feed, obtained from Wayne Feed Division, Continental Grain Company, Chicago, U.S.A., was composed of 4.0% crude fat (min.), 4.5% crude fibre (max.), and 24% crude protein (min.).

C.1 Administration of drugs

All animals were given an intraperitoneal (i.p.) injection of 0.05, 0.10 or 0.25 mmol/kg of the drug of interest. The drugs were dissolved in physiological saline or corn oil prior to administration. The N-ethoxycarbonyl analogues were not soluble in physiological saline solution and were therefore taken up in corn oil. All other drugs and amines were available as salts and were soluble in physiological saline.

C.2 Sample collection and storage

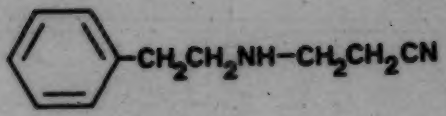
All animals were killed by rapid cervical dislocation at a specified period of time after a single i.p. injection. For time-concentration studies, rats were killed at 5, 15, 30, 60, 120 and 240 min after i.p. administration. Brains were rapidly dissected out (the meninges and pineal gland removed) and immediately frozen solid in isopentane on dry ice. These tissues were stored at -50° until time of analysis.

D. Chemical Synthesis of Arylalkylamine Analogues

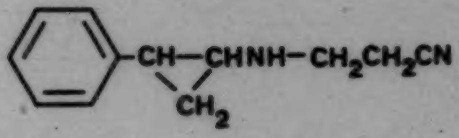
D.1 N-Cyanoethyl analogues of (±)-tranylcypromine, β-phenylethylamine and tryptamine

The chemical syntheses of (±)N-(2-cyanoethyl)tranylcypromine (CE-TCP), N-(2-cyanoethyl)phenylethylamine (CE-PEA), and N-(2-cyanoethyl)-tryptamine (CE-T) were conducted by Drs. T. W. Hall and R. G. Micetich (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta). All three N-cyanoethyl analogues were synthesized by the same general procedure; their chemical structures are illustrated in Figure 12. The primary parent amine was dissolved in benzene and refluxed with 4.7 equiv. of acrylonitrile for 24 h under an atmosphere of nitrogen. The mixture was cooled to room temperature, and the solvent and excess acrylonitrile were removed under reduced pressure (using an aspirator), to give the crude product. The pale yellow oil was purified by distillation under reduced pressure (0.1 mm Hg), which resulted in recovery of a small amount of the parent amine and the desired N-cyanoethyl analogue, obtained as colourless oil.

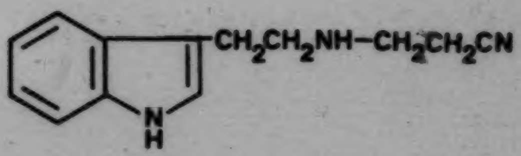
The distilled N-cyanoethyl analogue was dissolved in ether and



N-(2-cyanoethyl)phenylethylamine (CE-PEA)



N-(2-cyanoethyl)tranylcypromine (CE-TCP)



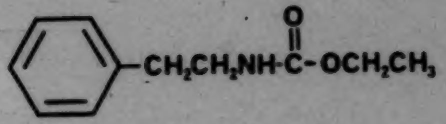
N-(2-cyanoethyl)tryptamine (CE-T)

Fig. 12. Chemical structures of the N-cyanoethyl analogues of β-phenylethylamine, tranylcypromine, and tryptamine.

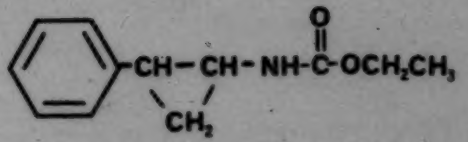
added dropwise to 1.25 equiv. of oxalic acid dihydrate dissolved in the mixture of ether and methanol (6.6:1). The gelatin-white precipitate was then collected by suction filtration to give an oxalate salt of the N-cyanoethyl analogue, or the hydrochloride salt was prepared by dissolving the distilled N-cyanoethyl analogue in anhydrous ether and gaseous hydrogen chloride bubbled into the solution. The oxalates or hydrochlorides gave characteristic mass spectra (MS), nuclear magnetic resonance (NMR) spectra, and infrared (IR) spectra consistent with their structures. Melting points were as follows: CE-TCP, 157-158° (dec.); CE-PEA, 176-177°; CE-T, 159-160° (dec.).

D.2 N-Ethoxycarbonyl analogues of (±)-tranylcypromine, β-phenylethylamine and (+)-amphetamine

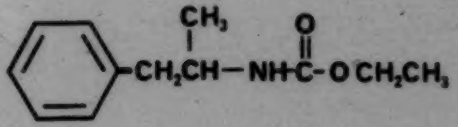
The chemical syntheses of the N-ethoxycarbonyl analogues of (±)-TCP, PEA and (+)-AM (Fig. 13) were conducted by Dr. T. J. Danielson (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta). The general procedure below was employed in the synthesis of the N-ethoxycarbonyl analogues. Ethyl chloroformate (1.6 ml) and sodium bicarbonate (2 g) were added to the primary parent amine (0.8 g) dissolved in distilled water. This mixture was shaken at room temperature for 1.5 h and was then extracted with ethyl acetate (1 x 50 ml). The ethyl acetate layer was retained and washed successively with water (20 ml) and 0.5 N HCl (20 ml). After drying over potassium carbonate, the ethyl acetate layer was filtered and evaporated to dryness to yield the N-ethoxycarbonyl analogue. The sample was further purified by distillation at reduced pressure and recrystallized from water and methanol. The N-ethoxycarbonyl analogues were characterized with elemental analy-



N-(ethoxycarbonyl)phenylethylamine (EthC-PEA)



N-(ethoxycarbonyl)tranylcypromine (EthC-TCP)



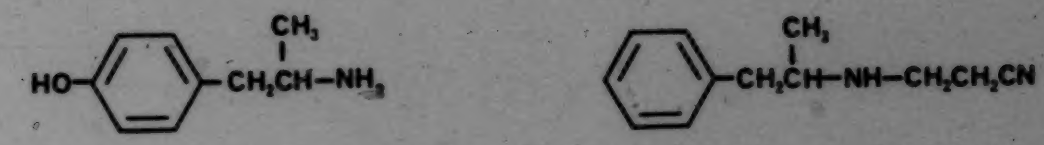
N-(ethoxycarbonyl)amphetamine (EthC-AM)

Fig. 13. Chemical structures of the N-ethoxycarbonyl analogues of β-phenylethylamine, tranylcypromine and amphetamine.

sis, nuclear magnetic resonance spectrometry (NMR), infrared spectrometry (IR), and mass spectrometry (MS). Melting points were as follows: (±)N-(ethoxycarbonyl)tranylcypromine (EthC-TCP), 45.0-47.0°; N-(ethoxycarbonyl)phenylethylamine (EthC-PEA), 33.0-34.1°; (±)N-(ethoxycarbonyl)amphetamine (EthC-AM), 43.6-45.0°. The melting point of EthC-PEA is in accordance with the reported value (Shriner and Child, 1952). However, the reported melting point of EthC-AM was significantly lower (23-23.5°) than that obtained by Dr. Danielson. Since Shriner and Child (1952) used only nitrogen analysis as the basis for characterizing their compounds, it is possible that impurity could have caused a lowering of the melting point in their sample.

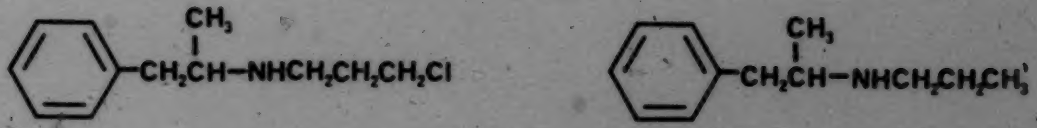
D.3 (±)N-n-Propylamphetamine

The chemical synthesis of (±)N-n-propylamphetamine (NPA) (Fig. 14) was conducted by Dr. F. M. Pasutto (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta). The following procedure was adopted for its synthesis. To 50 ml of dry ethanol, 150 mg of platinum dioxide was added. Hydrogenation of platinum dioxide was conducted in a Parr Hydrogenator for 30 min at 30 p.s.i. A solution containing phenylacetone (34 ml), n-propylamine (0.2 mol), and dry ethanol (100 ml) was added to the platinum fraction and hydrogenated overnight. The average uptake of hydrogen was 20 p.s.i. The solution was filtered and ethanol removed in vacuo. Ether (100 ml) was added to the residue and a saturated hydrogen chloride-ether solution added dropwise to form the hydrochloride salt. The sample was recrystallized from ethanol-ether. The compound gave MS and NMR spectra consistent with its structure.


HO
CH₃
CH₂CH–NH₂
CH₃
CH₂CH–NH–CH₂CH₂CN

para-hydroxyamphetamine
(p-OH-AM)

N-(2-cyanoethyl)amphetamine
(CE-AM)


CH₃
CH₂CH–NHCH₂CH₂CH₂Cl
CH₃
CH₂CH–NHCH₂CH₂CH₃

N-(3-chloropropyl)amphetamine
(CPA)

N-n-propylamphetamine
(NPA)

Fig. 14. Chemical structures of para-hydroxyamphetamine, N-(2-cyanoethyl)amphetamine, N-(3-chloropropyl)amphetamine, and N-n-propylamphetamine.

D.4 (±)N-2-Cyanoethyl and (±)N-3-chloropropyl analogues of amphetamine

The (±)N-2-cyanoethyl and the (±)N-3-chloropropyl analogues of amphetamine were synthesized by Drs. F. M. Pasutto and G. R. Jones (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta). Their chemical structures are illustrated in Figure 14. The cyanoethyl and the chloropropyl compound were prepared by reductive amination of phenylacetone with β-aminopropionitrile and 3-chloropropylamine respectively. Catalytic hydrogenation was effected by 10% palladium-charcoal (Pd-C) in ethanol. The compounds gave characteristic MS and NMR spectra.

E. Analysis of Bioactive Arylalkylamines and Analogues in Rat Brain

E.1 Aqueous pentafluorobenzoylation of amphetamine (AM) and N-alkylated analogues, para-hydroxyamphetamine (p-OH-AM), β-phenylethylamine (PEA), N-(2-cyanoethyl)phenylethylamine (CE-PEA) and N-(2-cyanoethyl)tranylcypromine (CE-TCP)

For the analyses of AM, CE-AM, CPA, NPA, p-OH-AM, PEA, CE-PEA and CE-TCP in rat brain, the weighed brain samples were homogenized in 5 volumes of ice-cold 0.4 N perchloric acid ($HClO_4$), and homogenates were transferred to Corex® 15 ml centrifuge tubes and centrifuged at 12,000 x g for 15 min at 0-4°. Two ml of the supernatant were retained for analysis. To the supernatant 1 μg of p-chlorophenylethylamine (p-Cl PEA) or 4 μg of 2,4-dichlorophenoxypropylamine (DCPPA) was added as internal standard. This was followed by the addition of solid potassium bicarbonate ($KHCO_3$) to neutralize the acid. The insoluble precipitate of potassium perchlorate was removed by centrifugation at room temperature for 5 min. To the precipitate-free supernatant a small excess of solid sodium bicarbonate ($NaHCO_3$) was added followed by 4 ml of extracting-derivatizing mixture composed of ethyl acetate:acetonitrile:pentafluorobenzoyl chloride (PFBC) in ratio 9:1:0.01. Extraction and derivatization of the arylalkylamines and drugs were done by vortexing the aqueous and organic phases together for 5 min. Centrifugation was performed at 3000 r.p.m. for 10 min to separate the phases. The organic phase was retained and taken to dryness under a stream of nitrogen. To the dried residue 100 µl of toluene and 200 µl of 1 N ammonium hydroxide (NH_4OH) were added. The mixture was vortexed for 15 sec and then microfuged for 60 sec. The toluene layer was retained and 1 µl was injected on the OV-101 capillary column for ECD-GLC analysis. Structures of the final derivatives were confirmed by gas-liquid chromatography-mass spectrometry (GLC-MS) (see Section III.E, mass spectra of derivatives, Fig. 28 to 33, 42 and 43).

This analytical procedure was a modification of the method published by Cristofoli et al. (1982b). A number of bioactive arylalkylamines and analogues were successfully analysed by this modified method. From a survey of the literature it appears that this is probably the first case where both primary and secondary bioactive amines were derivatized simultaneously in such mild aqueous conditions for ECD-GLC analysis. Although many reports of analyses of primary and secondary amines can be found, most were not in biological samples, and all were apparently carried out under anhydrous conditions, requiring heat in most cases for the derivatizing reaction.

E.2 Aqueous acetylation followed by anhydrous pentafluorobenzoylation of tranylcypromine (TCP) and β-phenylethylamine (PEA)

This analytical procedure was developed primarily because TCP could not be analysed by direct aqueous pentafluorobenzoylation. A co-chromatographing impurity from the brain could not be separated from TCP under these conditions. The analytical protocol developed in collaboration with D. R. Hampson (Hampson et al., 1984b) was as follows: brain samples were homogenized in 5 volumes of ice-cold 0.4 N $HClO_4$ and the brain homogenates were centrifuged at 12,000 x g for 15 min at 0-4°. To 2 ml of supernatant, internal standard (1 µg p-Cl-PEA) was added. The supernatant was neutralized with solid $KHCO_3$ and shaken with 5 ml of the ion-pairing compound DEHPA (2.5% v/v in chloroform) for 5 min. After centrifugation at 3000 r.p.m. for 5 min, the bottom layer was retained. To this, 2.5 ml of 0.5 N HCl was added and the mixture was vortexed for 5 min and centrifuged (3000 r.p.m. for 5 min). The top acid layer was retained, neutralized with solid $NaHCO_3$ and acetylated with 1/10 volume of acetic anhydride (Martin and Baker, 1976, 1977). When acetylation was complete, ethyl acetate was added and the mixture was vortexed for 5 min. Centrifugation was performed at 3000 r.p.m. for 5 min to separate the two phases. The ethyl acetate layer was taken to dryness under a stream of nitrogen, and the residue was heated for 1 h at 80° in 100 µl of toluene containing 2 µl of PFBC. After cooling, the mixture was washed with 200 µl of 1 N NH_4OH. The toluene layer was retained and an aliquot of this was taken for ECD-GLC analysis. Tranylcypromine and PEA were simultaneously analysed in the same sample of whole brain tissue with this procedure. Structures of the final derivatives were confirmed by GLC-MS (see Section III.E; mass spectra of derivatives, Fig. 39 to 41).

E.3 Aqueous trichloroacetylation of tranylcypromine (TCP) and β-phenylethylamine (PEA)

Yet another analytical procedure was developed for the analysis of TCP and PEA. This method too had to be developed out of necessity. Tranylcypromine could not be analysed by aqueous pentafluorobenzoylation due to insufficient separation from an interfering substance present in brain. Although TCP could be analysed by aqueous acetylation followed by anhydrous pentafluorobenzoylation, the pro-drug CE-TCP could not be separated from TCP by this method. Thus a novel method was developed so that TCP could be analysed in brain tissue, free from interference, when the animal was treated with the pro-drug CE-TCP. The method of analysis was as follows: brain samples were homogenized in 5 volumes of ice-cold 0.4 N $HClO_4$, centrifuged (12,000 x g for 15 min) at 0-4°, and 2 ml of the supernatant were retained for analysis. Internal standard (1 μg p-Cl-PEA) and solid $KHCO_3$ were added to the supernatant and the subsequent potassium perchlorate formed was removed by centrifugation. The supernatant was basified to pH 7.8 with solid $NaHCO_3$, and 1/10 volume of derivatizing reagent trichloroacetic anhydride (TCAA) added. The mixture was left to stand at room temperature for 15-20 min or until effervescence ceased. Excess $NaHCO_3$ was added to neutralize the trichloroacetic acid, produced as a biproduct of the reaction. Ethyl acetate (4 ml) was then added to the neutralized solution, and the mixture was vortexed for 5 min. The two phases were separated by centrifugation, and the organic layer was transferred to clean dry test tubes and evaporated to dryness under a stream of nitrogen. Toluene (100 μl) was then added to the dried residue and the mixture washed with 200 μl of distilled water. This was achieved by first vortexing for 15 sec and then microfuging in microfuge tubes for 60 sec. The toluene layer was retained and 0.2-1.0 µl injected onto an OV-1 capillary column in an ECD-GLC instrument. Structures of the final derivatives were confirmed by GLC-MS (see Section III.E; mass spectra of derivatives, Fig. 34 and 35).

Although trichloroacetyl derivatives have been employed by analysts to impart good ECD-GLC properties, it has not been the practice to prepare them in aqueous conditions. The above procedure is novel in this respect, as well as being a rapid and sensitive method for the simultaneous analysis of TCP and PEA (Baker et al., 1984a).

E.4 Anhydrous pentafluoropropionylation of the N-ethoxycarbonyl analogues of tranylcypromine, β-phenylethylamine and amphetamine

The previous methods employing pentafluorobenzoylation or trichloroacetylation did not impart good GLC properties or sensitivity to the N-ethoxycarbonyl analogues. Hence a method had to be found for analyses of these analogues in order to assess their activity and possible role as pro-drugs of AM, TCP or PEA. The following analytical protocol was found to give the best results. Brain samples were weighed and homogenized in 5 volumes of ice-cold 0.4 N $HClO_4$ and centrifuged to remove the protein precipitate. To 2 ml of supernatant, DCPPA (4 µg) was added as internal standard, followed by solid $KHCO_3$. The insoluble precipitate of potassium perchlorate was discarded after centrifugation. The N-ethoxycarbonyl analogues were extracted with ethyl acetate (4 ml) and the aqueous and organic layers were separated by centrifugation. The organic layer was transferred to clean, dry test tubes and subsequently taken to dryness under a stream of nitrogen. To the dried residue 25 µl of ethyl acetate and 75 µl of derivatizing reagent (PFPA) were added.

The mixture was reacted in a block-heater at 60° for 30 min. After cooling for 5 min, 300 µl of glass-distilled toluene was added, followed by 3 ml of saturated solution of sodium borate. The tubes were shaken briefly for 15 sec and centrifuged at 3000 r.p.m. for 1 min. The upper organic layer was retained and an aliquot (0.2-1 µl) injected on the OV-1 capillary column for ECD-GLC analysis. Structures of final derivatives were confirmed by GLC-MS (see Section III.E; mass spectra of derivatives, Fig. 36 to 38). The reason for employing a saturated solution of sodium tetraborate is as follows: the excess derivatizing reagent rapidly hydrolyses to the acid and partitions into the aqueous phase whereas the lipophilic PFP-derivative remains in the organic phase (Martin *et al.*, 1984). This then functions as selective partitioning and also as a "cleaning" procedure for the sample.

E.5 Anhydrous pentafluoropropionylation of tryptamine (T) and N-(2-cyanoethyl)tryptamine (CE-T)

Pentafluorobenzoylation or trichloroacetylation did not impart good GLC properties to the indoleamines tryptamine (T) or N-(2-cyanoethyl)-tryptamine (CE-T). Although aqueous acetylation followed by anhydrous pentafluoropropionylation did give sensitive and reproducible results, unfortunately T could not be separated from CE-T by this method. Hence a method was required to analyse T and CE-T, free of contamination from each other or interference from other impurities in the brain. Anhydrous pentafluoropropionylation was found to give sensitive derivatives of T and CE-T, and both compounds could be analysed simultaneously in the same piece of brain tissue. The analytical procedure developed follows. The brain samples were homogenised in ice-cold 0.4 N $HClO_4$ and, after centrifugation, 2 ml of the supernatant were retained. To this, internal standard (500 ng 5-methyltryptamine [5-MT]) and solid $KHCO_3$ were added. The precipitate of potassium perchlorate was removed after centrifugation. After adding excess solid $NaHCO_3$ to ensure a slightly basic environment, 4 ml of ethyl acetate was added to the supernatant. The mixture was vortexed for 5 min and centrifuged. The upper organic phase was transferred into clean, dry glass test tubes, and evaporated to dryness under a stream of nitrogen. To the dried residue, 25 µl ethyl acetate and 75 µl of PFPA were added, and the derivatization reaction was allowed to proceed for 30 min at 60°. After cooling, the reaction mixture was partitioned between 300 µl of toluene and 3 ml of saturated solution of sodium borate as described in the previous section of this thesis. The upper toluene layer was retained and 1-2 µl was injected onto the 3% OV-17 packed column for ECD-GLC analysis. Structures of the final derivatives were confirmed by GLC-MS (see Section III.E; mass spectra of derivatives, Fig. 44 and 45).

E.6 Analysis of the endogenous biogenic amines tryptamine (T) and 5-hydroxytryptamine (5-HT)

The biogenic amines T and 5-HT were analysed by a modification of the procedure of Baker *et al.* (1980). Brain samples were weighed and homogenized in 5 volumes of ice-cold 0.1 N $HClO_4$ containing 10 mg % EDTA. The homogenates were centrifuged and the supernatants retained. To 3 ml of the supernatant, 500 ng of the internal standard 5-MT were added, followed by solid $KHCO_3$ to neutralize the acid, and the potassium perchlorate precipitate formed was subsequently removed by centrifugation. Sodium phosphate buffer (pH 7.8, 0.25 M, 400 µl) and the liquid ion-pairing reagent DEHPA (2.5% v/v in chloroform, 4 ml) were then added. The mixture was vortexed for 5 min and the two phases separated by centrifugation. The upper aqueous layer was aspirated off, and to the bottom chloroform layer 0.5 N HCl (2.5 ml) was added. This mixture was vortexed for 5 min and the two layers were separated by centrifugation. The upper HCl layer was transferred to clean, dry test tubes and neutralized with solid $NaHCO_3$. To this mixture 300 µl of acetic anhydride was added and tubes were left to stand for 15-20 min or until the effervescence had ceased. Excess solid $NaHCO_3$ was added and the mixture extracted with 4 ml of ethyl acetate and centrifuged. The ethyl acetate layer was then transferred into clean, dry test tubes, and evaporated to dryness under a stream of nitrogen. The dried residue was reacted with 75 µl of PFPA and 25 µl ethyl acetate at 60° for 30 min. After cooling, the reaction mixture was partitioned between 300 µl of cyclohexane and 3 ml of a saturated solution of sodium tetraborate. The cyclohexane layer was retained and an aliquot (0.2-1.0 µl) injected on the OV-1 capillary column for sensitive ECD-GLC analysis.

E.7 Sensitivity of the methods of analyses

The on-column sensitivity for each bioactive amine was determined as the ECD response which was at least twice the "blank" response.

F. Monoamine Oxidase Assay

A modification of the method of Wurtman and Axelrod (1963) was employed for the assay of monoamine oxidase activity. Rat brains were homogenized in ice-cold isotonic KCl to give a 4% homogenate. The brain tissue homogenate (25 µl) was added to all tubes except blanks, to which only isotonic KCl (25 µl) was added. All tubes were then placed on ice, and to each tube 250 µl of 0.5 M sodium phosphate buffer (pH 7.4) was added. (For _in vitro_ assay, test drug was added to the tubes so that its final concentration was 4 µM, and the tubes were incubated at 37° for 10 min. Drugs available as salts were dissolved directly in isotonic KCl solution, while the N-ethoxycarbonyl analogues of PEA, AM and TCP were dissolved in dimethylsulphoxide [DMSO] and diluted in the KCl solution before addition to the assay system.) Suitably diluted solutions of radiolabelled ^{14}C-5-HT (substrate for MAO-A) or ^{14}C-PEA (substrate for MAO-B) in 25 µl aliquots were added to all tubes (to give final concentrations of PEA and 5-HT of 35 µM), which were then incubated at 37° for 20 min. After the tubes were cooled to room temperature, 2 N HCl (200 µl) was added to each tube to stop the reaction. Toluene (6 ml) was added to all the tubes and mixtures vortexed for 3 min. The two phases were separated by centrifugation at 2500 r.p.m. for 5 min. The tubes were then kept in the freezer at -70° for 20 min or until the aqueous layer was frozen. The toluene layer was decanted into scintillation vials containing 9 ml of scintillation fluid. The radioactivity (c.p.m.) of each sample was measured in the liquid scintillation spectrometer. The following equation was employed to determine % MAO inhibition:

$$\%\ \text{MAO inhibition} = 100 - \frac{\text{corrected sample (c.p.m.)}}{\text{mean corrected control (c.p.m.)}} \times 100$$

The corrected sample and control values were obtained by subtracting the blank values.

G. Statistical Analyses

Statistical analyses of samples were performed on a programmable TI-55-II Texas Instruments calculator or a Hewlett Packard (HP) 86 microcomputer with an HP 9130A flexible disc drive coupled to an HP 82905B printer. The standard mean error (S.E.M.) is represented by error bars on all figures. Student's *t*-test for independent means was employed for pairwise comparisons between the groups. A one-tailed probability distribution was used for all statistical analyses and the general convention of $p < 0.05$ was employed for establishment of statistical significance.

III. RESULTS

A. Brain Concentrations of Bioactive Arylalkylamines and Analogues

A.1 Amphetamine and N-alkylated analogues

A novel method of analysis was developed for a rapid and sensitive assay of amphetamine (AM) and its N-alkylated analogues in the same piece of brain tissue (Section II.E.1). Analysis was performed using ECD-GLC with a capillary column after reaction of compounds with PFBC under aqueous conditions. The brain concentrations of AM and its N-alkylated analogues (after an intraperitoneal [i.p.] administration of 0.05, 0.10 or 0.25 mmol/kg of the N-alkylated analogue) are illustrated in Table IV.

The results indicate that all three N-alkylated analogues of AM were converted to AM, but the N-(2-cyanoethyl)amphetamine (CE-AM) gave the highest amounts of AM in the brain. The level of AM was significantly higher than the level of CE-AM in the brain samples. The analogues N-(3-chloropropyl)amphetamine (CPA) and N-_n_-propylamphetamine (NPA) were converted to about equal amounts of AM in the brain samples, but the levels of CPA and NPA in the respective brain samples were much higher than their corresponding AM levels, in contrast to what was observed with CE-AM.

A.2 Time-concentration profile of amphetamine and its metabolite para-hydroxyamphetamine

The concentration of AM and its metabolite _p_-OH-AM were measured in rat brain at time 5, 15, 30, 60, 120 and 240 min after 0.1 mmol/kg i.p.

Table IV. Concentrations of N-alkylated amphetamines and of amphetamine in rat brain at 1 h after i.p. dose.

	Dose of N-alkylated amphetamine (mmol/kg)	Concentration of N-alkylated amphetamine (nmol/g)	Concentration of amphetamine (nmol/g)
	CE-AM	CE-AM	
n = 6	0.05	7.44 (± 0.59)	23.70 (± 3.18)
	0.10	12.23 (± 1.54)	43.70 (± 6.52)
	0.25	57.44 (± 6.33)	150.37 (± 15.40)
	CPA	CPA	
n = 6	0.05	9.17 (± 2.60)	4.15 (± 0.66)
	0.10	16.36 (± 4.25)	12.22 (± 1.93)
	0.25	73.28 (± 16.07)	33.92 (± 6.74)
	NPA	NPA	
n = 6	0.05	7.34 (± 1.46)	3.41 (± 0.52)
n = 5	0.10	19.49 (± 3.67)	9.41 (± 1.19)
n = 5	0.25	80.79 (± 12.54)	39.19 (± 3.48)
	AM		
n = 6	0.05	--	39.5 (± 3.41)
	0.10	--	157.3 (± 19.86)

Results represent mean ± S.E.M.

dose of AM. Analyses of AM and p-OH-AM were performed simultaneously with ECD-GLC after aqueous pentafluorobenzoylation (Section II.E.1). The time-concentration profiles of AM and p-OH-AM are illustrated in Figures 15 and 16 respectively. The results indicate the time at which concentration reaches a maximum (t_{max}) was 30 min for AM, whereas the t_{max} for p-OH-AM was 60 min. The areas under the curve (AUC_{240}^{0}) of the time-concentration graphs for AM (Fig. 15) and p-OH-AM (Fig. 16) were determined to be 17.55 and 0.43 μmol min/g respectively. The maximum concentration (C_{max}) of AM and p-OH-AM in brain (after administration of AM) was 169.9 ± 40 nmol/g (mean ± S.E.M.) and 2.8 ± 0.9 nmol/g respectively, and the elimination half-lives ($t_{1/2}$s) of AM and p-OH-AM from brain were determined to be 43.0 and 100.3 min respectively.

A.3 Time-concentration profile of N-(2-cyanoethyl)amphetamine and its metabolites amphetamine and para-hydroxyamphetamine

Aqueous pentafluorobenzoylation (Section II.E.1) was employed in the simultaneous analysis of CE-AM, AM, and p-OH-AM in brain tissue after administration of CE-AM (0.1 mmol/kg i.p.). A gas chromatogram of a brain sample from a rat treated with CE-AM and sacrificed at 1 h is depicted in Figure 19. In Figures 17 and 18 are illustrated time-concentration profiles of CE-AM, AM and p-OH-AM respectively after administration of CE-AM. The t_{max}s of CE-AM, AM and p-OH-AM were determined to be 5, 15 and 15 min respectively, and C_{max}s at these times for CE-AM, AM and p-OH-AM were 118.8 (± 12.5), 94.6 (± 7.1), and 10.8 (± 1.7) nmol/g respectively. Areas under the curve (AUC_{240}^{0}) for CE-AM and AM were determined to be 3.79 and 8.66 μmol min/g respectively. The AUC_{120}^{0} of p-OH-AM was 0.35 μmol min/g.

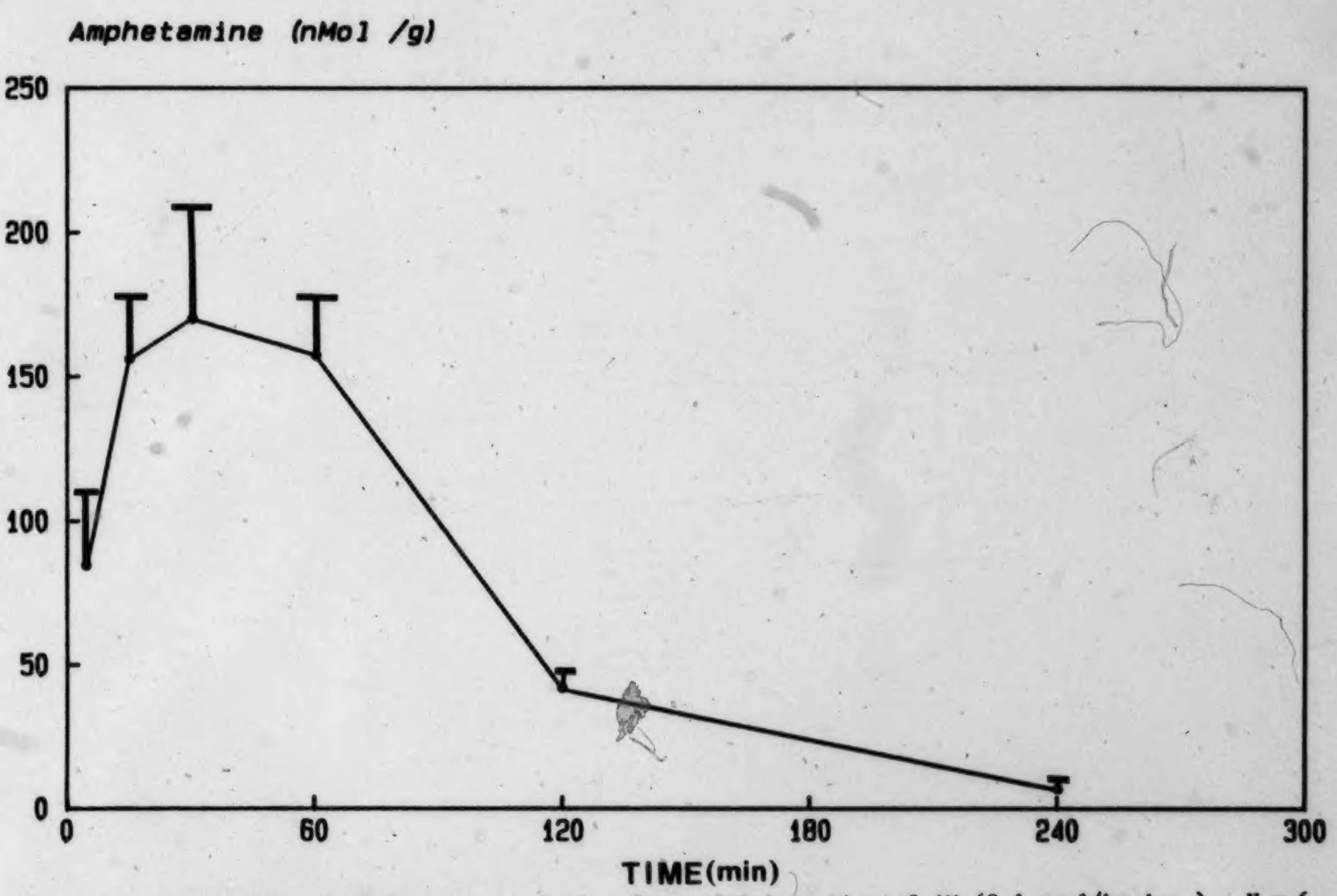


Fig. 15. Levels of amphetamine in rat brain after administration of AM (0.1 mmol/kg i.p.). N = 6. Error bars = S.E.M.

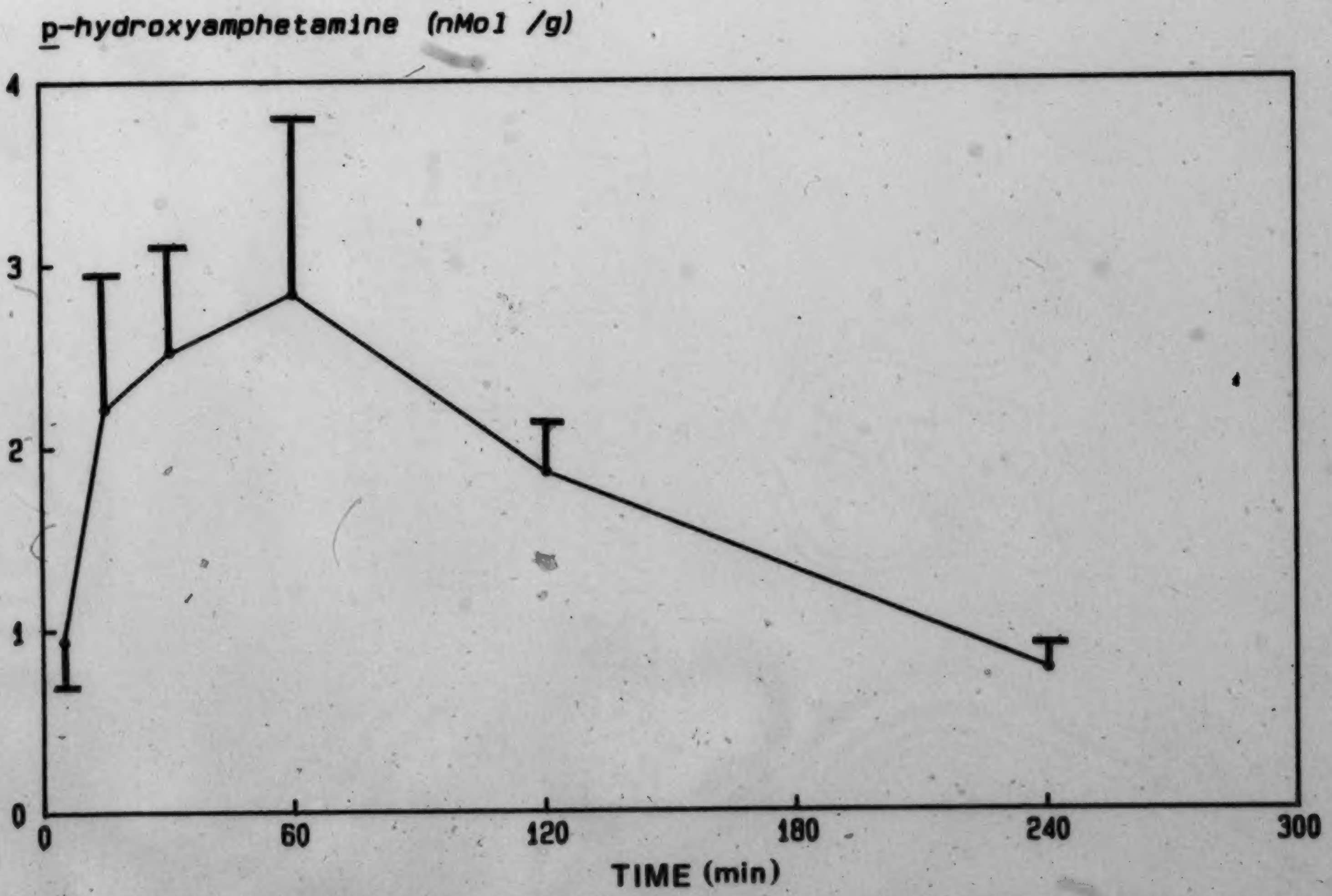


Fig. 16. Levels of para-hydroxyamphetamine in rat brain after administration of AM (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.

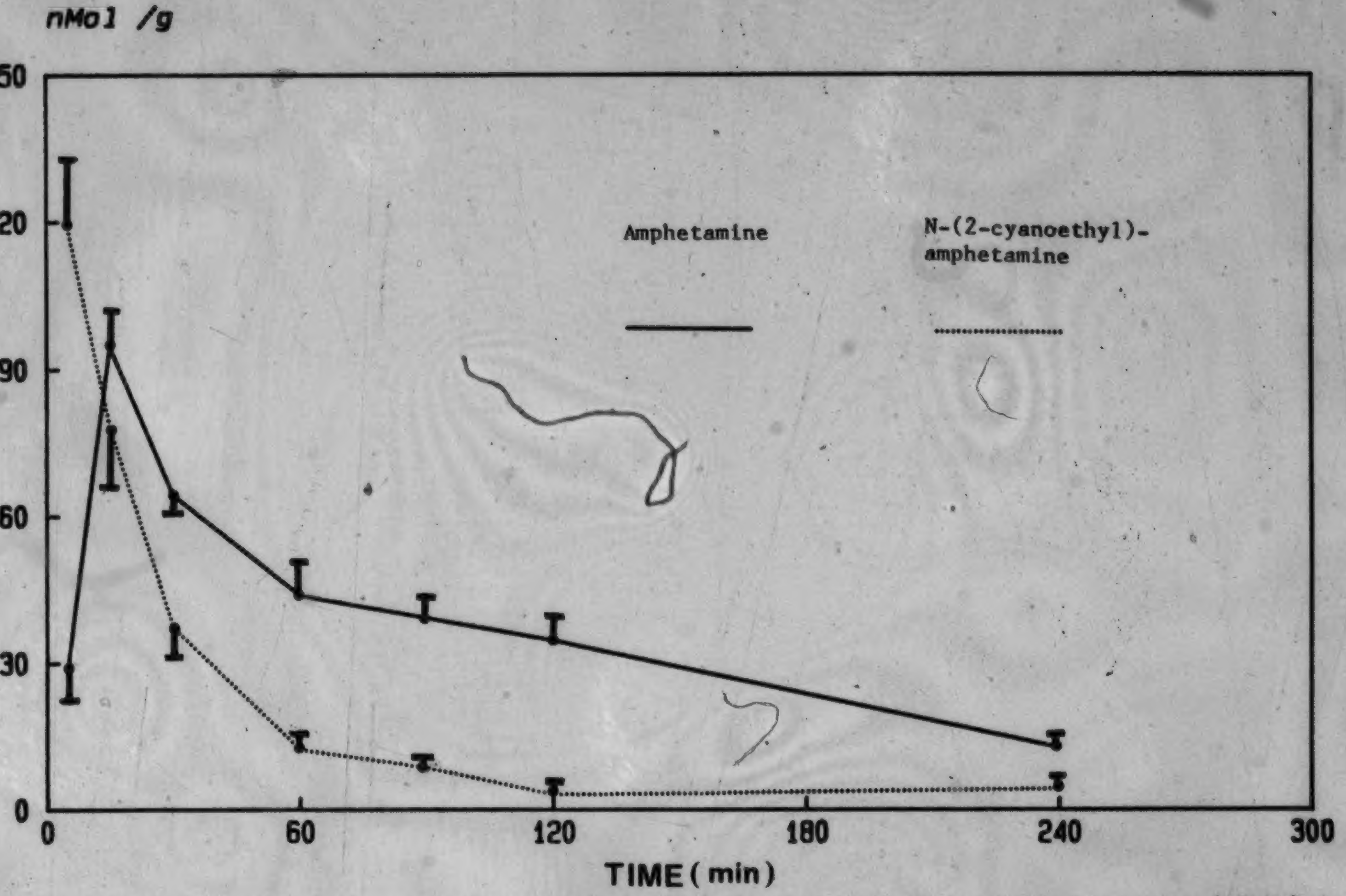


Fig. 17. Time-concentration profile of amphetamine (AM) and N-(2-cyanoethyl)amphetamine (CE-AM) in rat brain after administration of CE-AM (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.

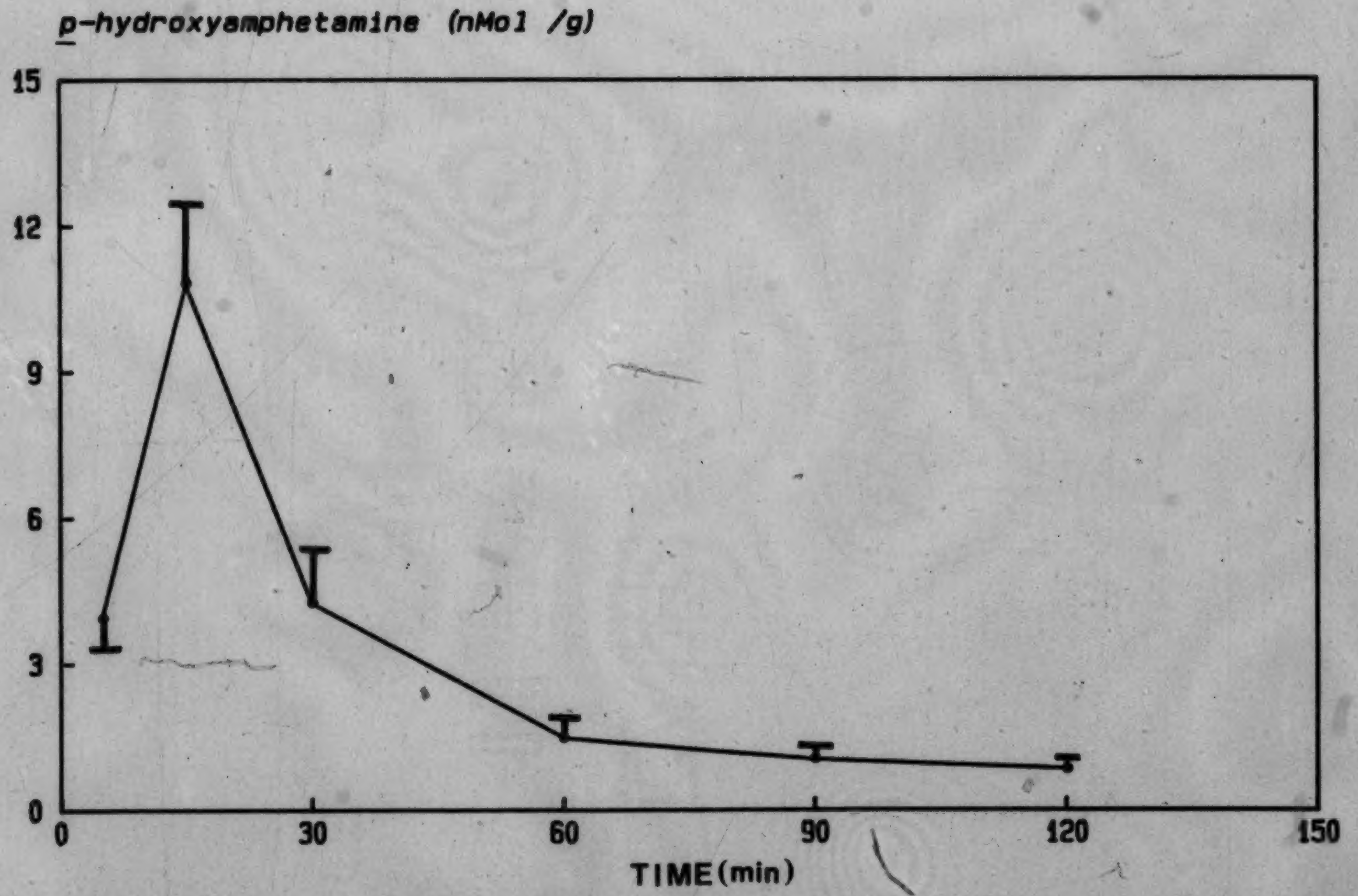


Fig. 18. Levels of para-hydroxyamphetamine in the rat brain after administration of CE-AM (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.

From Figure 17, it can be observed that CE-AM entered the brain very rapidly, reaching a maximum within the first five minutes. However, CE-AM is also rapidly removed from the brain. In the time period from 5 min to 120 min, the removal of CE-AM from brain was exponential and the half-life ($t_{1/2}$) in this phase was only 21.5 min. The concentration of CE-AM stabilized after 120 min. The concentration of AM reached a maxima at 15 min, and AM levels remained higher than CE-AM at all times after 15 min. The elimination half-life ($t_{1/2}$) of AM and p-OH-AM were determined to be 100.3 min and 75.2 min respectively.

A.4 Time-concentration profile of tranylcypromine

Aqueous acetylation followed by pentafluorobenzoylation (Section II.E.2) (Hampson et al., 1984b) was employed in the analysis of tranylcypromine (TCP) in rat brain after i.p. administration of TCP (0.1 mmol/kg). A time-concentration profile of TCP in rat brain is illustrated in Figure 20. The t_{max} and C_{max} of TCP in rat brain were found to be 30 min and 125.3 (± 11.5) nmol/g respectively. The area under the curve (AUC_{240}^{0}) was 15.8 μmol min/g and the elimination half-life ($t_{1/2}$) of TCP from brain was 100.3 min.

A.5 Time-concentration profile of N-(2-cyanoethyl)tranylcypromine and its metabolite tranylcypromine

The concentration of N-(2-cyanoethyl)tranylcypromine (CE-TCP) in the rat brain was measured after aqueous pentafluorobenzoylation (Section II.E.1). A novel procedure employing aqueous trichloroacetylation (Baker et al., 1984a) was used for the simultaneous analysis of TCP and PEA in brain tissue (Section II.E.3). Analysis of CE-TCP and TCP was

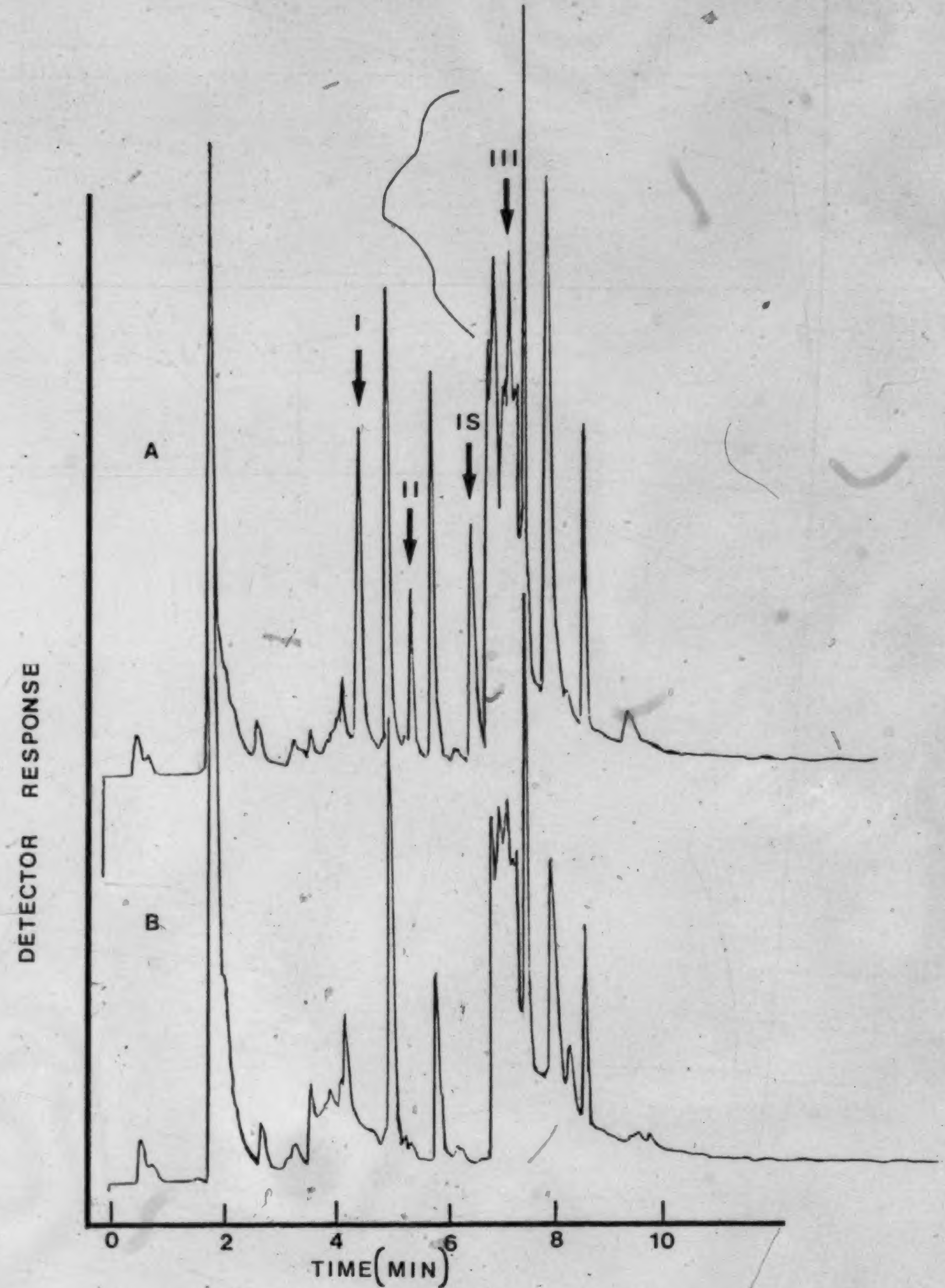


Fig. 19. A gas chromatogram of a brain sample from a rat treated with N-(2-cyanoethyl)amphetamine (0.1 mmol/kg i.p.) and sacrificed at 1 h (A), and a control brain sample (B). The peaks are pentafluorobenzoyl derivatives of: amphetamine (I), N-(2-cyanoethyl)amphetamine (II), para-hydroxyamphetamine (III), and 2,4-dichlorophenoxypropylamine (I.S.).

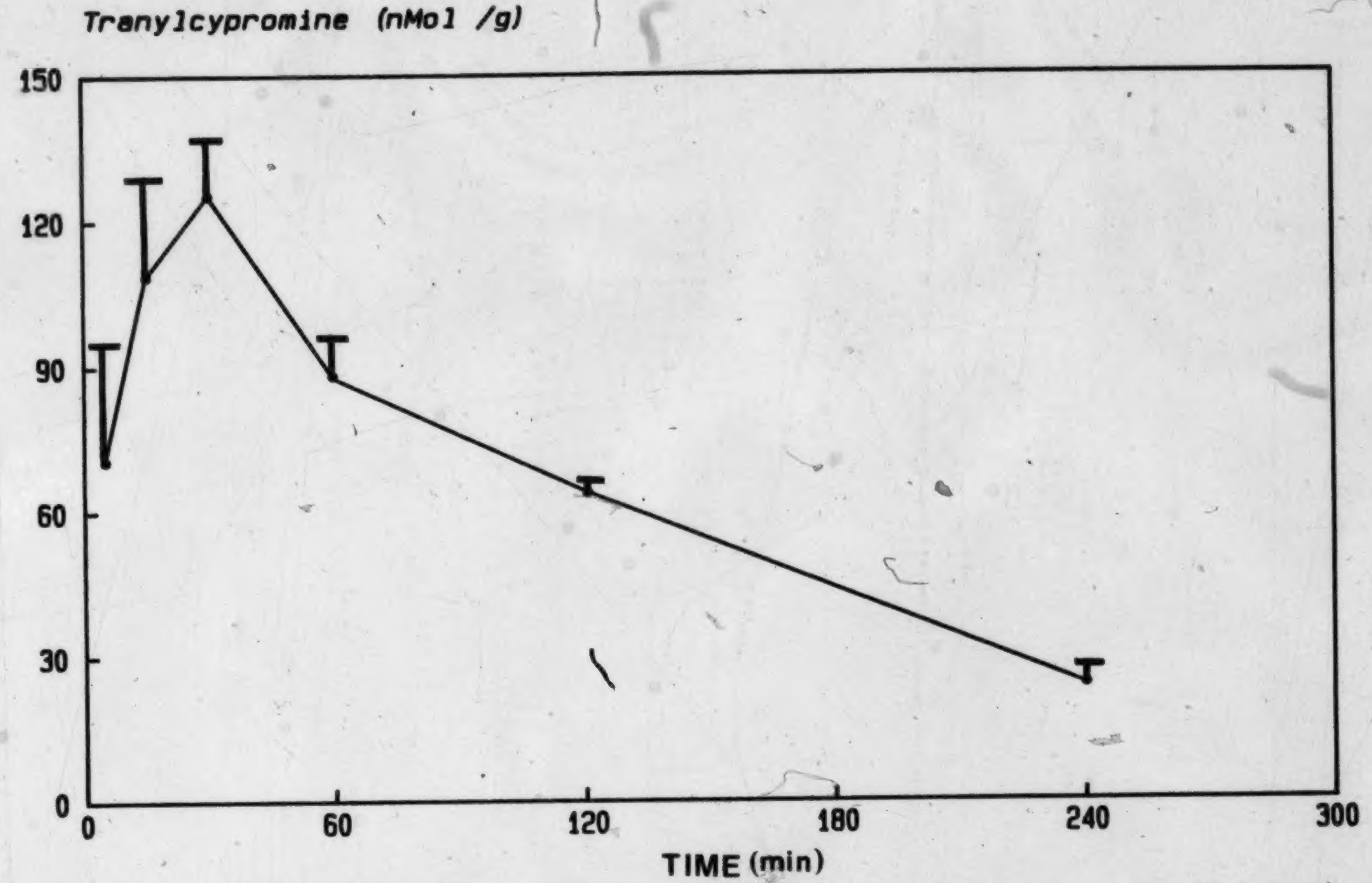


Fig. 20. Levels of tranylcypromine (TCP) in rat brain after administration of TCP (0.1 mmol/kg i.p.). N = 6. Error bars = S.E.M.

performed on a GLC equipped with a capillary column and an electron-capture detector. Time-concentration profiles of CE-TCP and TCP in rat brain after i.p. administration of CE-TCP are illustrated in Figure 21. The levels of TCP in brain remain higher than CE-TCP at all times. This suggests good conversion of CE-TCP to TCP. Areas under the curve (AUC_{240}^{0}) for CE-TCP and TCP were determined from Figure 21 to be 1.15 µmol min/g and 1.55 µmol min/g respectively. The brain elimination half-lives ($t_{1/2}$s) of CE-TCP and TCP after i.p. administration of CE-TCP were determined to be 300.9 min and 150.5 min respectively (calculation based on log data from the elimination portion of the curve, i.e. from 120 min onwards).

A.6 Preliminary investigation of the N-ethoxycarbonyl analogues of amphetamine, β-phenylethylamine and tranylcypromine

Preliminary studies were conducted with the N-ethoxycarbonyl analogues of AM, PEA, and TCP in rat brain to assess their role as possible pro-drugs of AM, PEA or TCP (Baker et al., 1984c). Anhydrous pentafluoropropionylation was employed for the sensitive ECD-GLC analyses of the N-ethoxycarbonyl analogues (Section II.E.4). The concentrations of AM, PEA and TCP in brain tissue were measured by the method of Hampson et al., 1984a,b), employing aqueous acetylation followed by pentafluorobenzoylation. The results are illustrated in Table V. In this study, brain concentrations of AM and TCP in rats 1 h after administration of the N-ethoxycarbonyl analogues (0.1 mmol/kg i.p.) were calculated to be 9.55 (± 1.63) nmol/g (mean ± S.E.M.) and 8.95 (± 1.28) nmol/g respectively. The concentrations of the N-ethoxycarbonyl analogues in the same brain tissues were somewhat lower: 4.15 (± 0.58) nmol/g and 4.83 (±

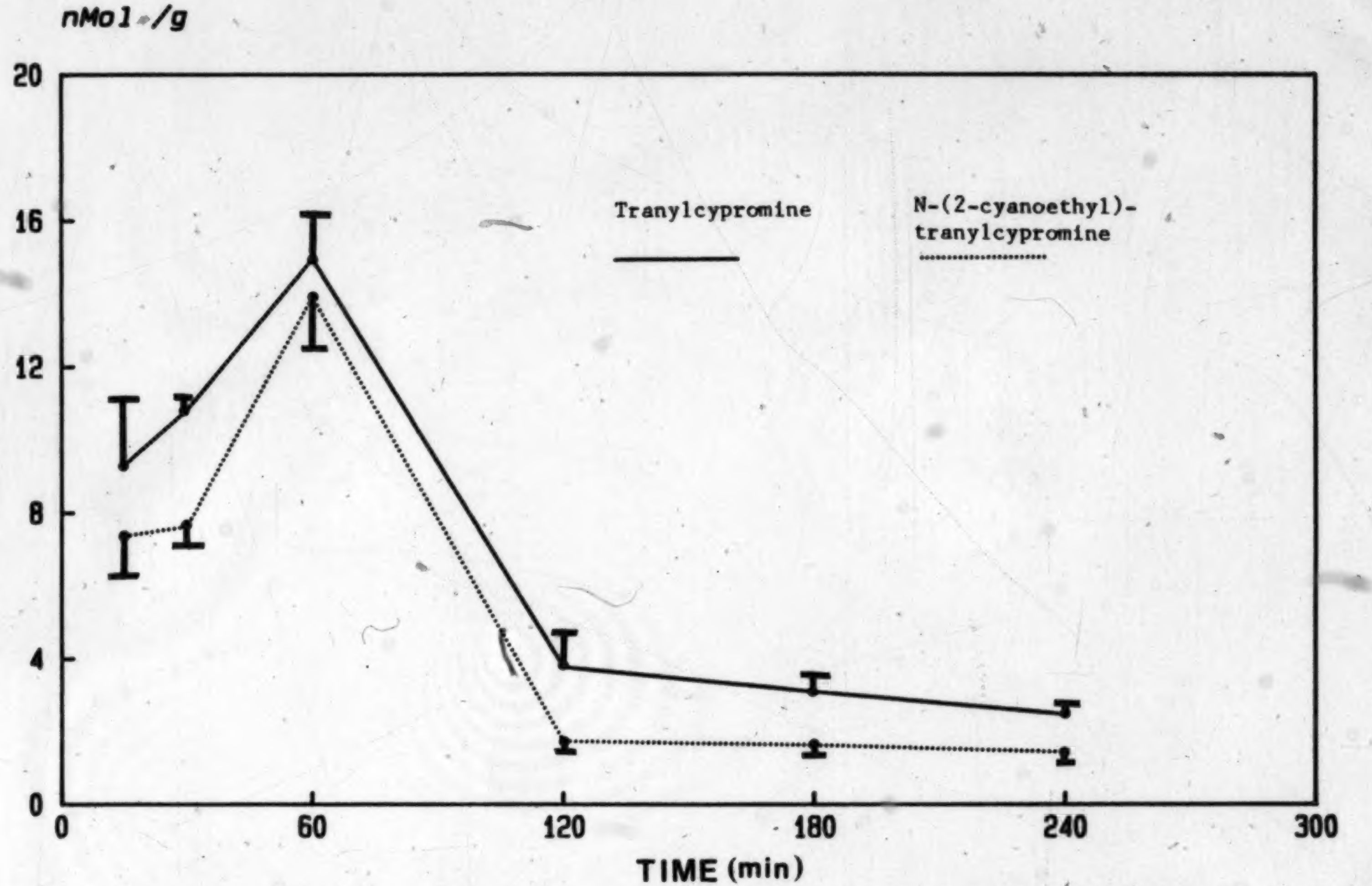


Fig. 21. Levels of tranylcypromine (TCP) and N-(2-cyanoethyl)tranylcypromine (CE-TCP) in rat brain after administration of CE-TCP (0.1 mmol/kg i.p.). N = 5-6. Error bars = S.E.M.

0.68) nmol/g for N-(ethoxycarbonyl)amphetamine (EthC-AM) and N-(ethoxycarbonyl)tranylcypromine (EthC-TCP) respectively. In a preliminary *in vivo* investigation of N-(ethoxycarbonyl)phenylethylamine (EthC-PEA), this drug was injected at a dose of 0.5 mmol/kg and the rats were sacrificed 1 h later. This larger dose was used so that the PEA formed could be measured; PEA, unlike AM or TCP, is rapidly metabolized by MAO. Under these conditions, brain levels of EthC-PEA and PEA were 64.2 (± 8.2) nmol/g and 190 (± 36) pmol/g respectively. In another group of rats in which PEA HCl (0.5 mmol/kg) was injected, brain levels of PEA were 835 (± 170) pmol/g. As the normal brain levels of PEA are 10 to 15 pmol/g, the administration of the carbamate analogue may represent a method for selectively increasing brain concentrations of this amine without affecting levels of other biogenic amines.

A.7 Preliminary investigation of the N-cyanoethyl analogues of β-phenylethylamine (PEA) and tryptamine (T)

The N-cyanoethyl analogues of AM and TCP were found to have good pro-drug properties, forming AM and TCP in brain in pharmacologically active concentrations. Hence preliminary studies were conducted to investigate possible pro-drug properties of N-(2-cyanoethyl)phenylethylamine (CE-PEA) and N-(2-cyanoethyl)tryptamine (CE-T). The first objective was to develop analytical methods for the quantitation of CE-PEA and CE-T in brain. Aqueous pentafluorobenzoylation was found to give the best results for the simultaneous analysis of CE-PEA and PEA (Section II.E.1), and anhydrous pentafluoropropionylation for analysis of CE-T and T (Section II.E.5) in the same piece of brain tissue, employing ECD-GLC. Because control brain levels of T are < 3.5 pmol/g (thus

Table V. Concentrations of the N-ethoxycarbonyl analogues of amphetamine (AM) and tranylcypromine (TCP) and of AM and TCP in rat brain at 1 h after an i.p. dose.

	Dose of N-ethoxycarbonyl analogue (mmol/kg)	Concentration of N-ethoxycarbonyl analogue (nmol/g)	Concentration of active metabolite (nmol/g)
	EthC-AM	EthC-AM	AM
n = 6	0.1	4.15 (± 0.58)	9.55 (± 1.63)
	EthC-TCP	EthC-TCP	TCP
n = 6	0.1	4.83 (± 0.68)	8.95 (± 1.28)

Results represent mean ± S.E.M.

making analysis of control levels of this amine by ECD-GLC impossible), and since PEA and T (unlike AM or TCP) are rapidly metabolized by MAO, the rats were pre-treated with pargyline (60 mg/kg i.p.), an MAO inhibitor, 45 min prior to administration of CE-PEA or CE-T (dose 0.1 mmol/kg i.p.) so that the PEA or T formed could be measured. The rats were killed 1 h after administration of CE-PEA or CE-T. Under these conditions, brain levels of CE-PEA and PEA were 7.93 (± 1.32) nmol/g (mean ± S.E.M.) and 26.28 (± 0.99) nmol/g respectively, and brain levels of CE-T and T were 69.09 (± 5.39) nmol/g and 4.73 (± 0.18) nmol/g respectively. The brain levels of PEA and T in rats treated with pargyline only (i.e. no pro-drug) were 1.09 (± 0.13) nmol/g and 21.76 (± 2.2) pmol/g respectively. As the normal brain levels of PEA and T are only 10 to 15 pmol/g and < 3.5 pmol/g respectively, the administration of CE-PEA or CE-T results in substantial elevation of PEA and T over control values and even over values obtained in rats after injection of pargyline. Thus administration of the N-cyanoethyl analogues may represent a method for selectively increasing brain concentrations of PEA or T without affecting levels of other biogenic amines. The N-cyanoethyl analogue of PEA appears to be a good source of PEA, with concentrations of PEA being greater than CE-PEA in brain. The results are depicted in Table VI. Detailed time studies by T. S. Rao in our laboratories have now demonstrated that CE-PEA, even in the absence of an MAO inhibitor, does cause elevated, sustained increases in brain levels of PEA (personal communication).

A.8 Linearity and Sensitivity Studies

Standard curves were carried through with each analytical run, and

Table VI. Concentrations of the N-cyanoethyl analogues of β-phenylethylamine (PEA) and tryptamine (T) and of PEA and T in rat brain at 1 h after i.p. dose (pretreated with pargyline, 60 mg/kg i.p.).

	Dose of the N-cyanoethyl analogue (mmol/kg)	Concentration of N-cyanoethyl analogue (nmol/g)	Concentration of amine (nmol/g)
	CE-PEA	CE-PEA	PEA
n = 6	0.1	7.93 (± 1.32)	26.28 (± 0.99)
n = 6	CE-T	CE-T	T
	0.1	69.09 (± 5.39)	4.73 (± 0.18)

Results represent mean ± S.E.M.

all assays were linear for at least a 50-fold range of concentrations of the compound of interest. The on-column sensitivity (defined as twice the blank value) of all methods developed for analysis of the bioactive amines and analogues is shown in Table VII.

B. Inhibition of Monoamine Oxidase

The cyanoethyl and ethoxycarbonyl analogues of the bioactive amines were tested for inhibitory activity against MAO-A and MAO-B *in vitro* (at a concentration of 4 μM), employing a modification of Wurtman and Axelrod's procedure (1963) (Section II.F). The results are shown in Table VIII. Of all the bioactive amine analogues tested, only CE-TCP had MAO-inhibiting activity of over 80% against MAO-A and MAO-B. This was comparable to the activity of TCP under the same conditions. The other three cyanoethyl analogues were considerably less active. Placed in descending order of activity, CE-T was more active than CE-AM which in turn was more active than CE-PEA against both MAO-A and MAO-B. The ethoxycarbonyl analogues of AM, PEA and TCP had little or no MAO-inhibiting activity.

A preliminary study of EthC-TCP showed metabolism of this compound to TCP with detectable quantities of TCP in rat brain (indicated in Table V). Since TCP is a strong inhibitor of MAO, the study on the N-ethoxycarbonyl analogue of TCP was extended to include an investigation of MAO activity (Baker *et al.*, 1984c). The results are listed in Table IX. The concentrations of EthC-TCP and of TCP equivalent to concentrations found *in vivo* (assuming that 1 g of brain is approximately equivalent to 1 ml) were tested *in vitro*. The results show that EthC-

Table VII. The maximum on-column sensitivity of all the methods developed in the analysis of the bioactive amines and analogues. Maximum sensitivity represents the amount of compound at which there is a signal-to-noise ratio of 2:1.

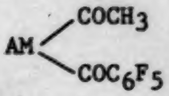
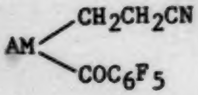
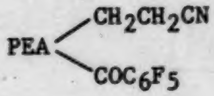
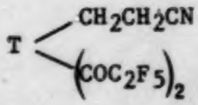
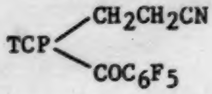
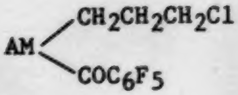
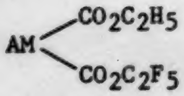
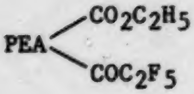
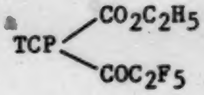
COMPOUND	DERIVATIZING REAGENT	DERIVATIVE	"ON-COLUMN" SENSITIVITY (ng)
AM	PFBC	$\mathrm{AM{-}COC_6F_5}$	0.01
AM	AA PFBC	 $\mathrm{AM}{<}^{\mathrm{COCH_3}}_{\mathrm{COC_6F_5}}$	0.01
CE-AM	PFBC	 $\mathrm{AM}{<}^{\mathrm{CH_2CH_2CN}}_{\mathrm{COC_6F_5}}$	0.025
CE-PEA	PFBC	 $\mathrm{PEA}{<}^{\mathrm{CH_2CH_2CN}}_{\mathrm{COC_6F_5}}$	0.01
CE-T	PFPA	 $\mathrm{T}{<}^{\mathrm{CH_2CH_2CN}}_{(\mathrm{COC_2F_5})_2}$	0.017
CE-TCP	PFBC	 $\mathrm{TCP}{<}^{\mathrm{CH_2CH_2CN}}_{\mathrm{COC_6F_5}}$	0.01
CPA	PFBC	 $\mathrm{AM}{<}^{\mathrm{CH_2CH_2CH_2Cl}}_{\mathrm{COC_6F_5}}$	0.08
EthC-AM	PFPA	 $\mathrm{AM}{<}^{\mathrm{CO_2C_2H_5}}_{\mathrm{CO_2C_2F_5}}$	0.017
EthC-PEA	PFPA	 $\mathrm{PEA}{<}^{\mathrm{CO_2C_2H_5}}_{\mathrm{COC_2F_5}}$	0.015
EthC-TCP	PFPA	 $\mathrm{TCP}{<}^{\mathrm{CO_2C_2H_5}}_{\mathrm{COC_2F_5}}$	0.020

Table VII. (cont'd)

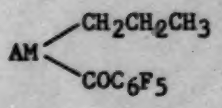
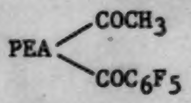
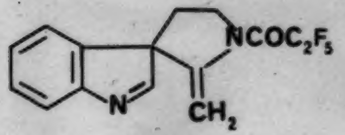
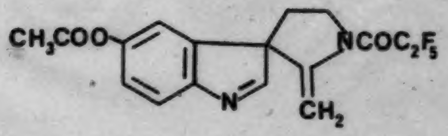
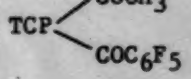
COMPOUND	DERIVATIZING REAGENT	DERIVATIVE	"ON-COLUMN" SENSITIVITY (ng)
NPA	PFBC	 $\mathrm{AM}<^{\mathrm{CH_2CH_2CH_3}}_{\mathrm{COC_6F_5}}$	0.03
PEA	PFBC	$\mathrm{PEA{-}COC_6F_5}$	0.01
PEA	AA PFBC	 $\mathrm{PEA}<^{\mathrm{COCH_3}}_{\mathrm{COC_6F_5}}$	0.005
PEA	TCAA	$\mathrm{PEA{-}COCCl_3}$	0.005
p-OH-AM	PFBC	$\mathrm{C_6F_5COO{-}AM{-}COC_6F_5}$	0.05
T	PFPA	$\mathrm{T{-}(COC_2F_5)_2}$	0.007
T	AA PFPA		0.01
5-HT	AA PFPA		0.01
TCP	AA PFBC	 $\mathrm{TCP}<^{\mathrm{COCH_3}}_{\mathrm{COC_6F_5}}$	0.005
TCP	TCAA	$\mathrm{TCP{-}COCCl_3}$	0.005

Table VIII. The percent inhibition of MAO-A and MAO-B *in vitro*. Concentration of drug used was 4 μM.

COMPOUND	% MAO-A INHIBITION	% MAO-B INHIBITION
CE-AM	28.9 (± 4.3)	8.9 (± 2.2)
CE-PEA	15.5 (± 2.4)	4.6 (± 1.7)
CE-T	51.0 (± 4.4)	25.1 (± 1.2)
CE-TCP	81.7 (± 3.1)	85.6 (± 1.2)
EthC-AM	nil	nil
EthC-PEA	nil	10.1 (± 0.9)
EthC-TCP	11.8 (± 2.7)	nil
TCP	87.4 (± 1.6)	94.2 (± 1.4)

Results represent mean ± S.E.M. N = 4-6.

TCP caused 11.7 (± 4.1)% inhibition (mean ± S.E.M.) of MAO-A and no inhibition of MAO-B, while TCP caused 83.9 (± 5.5.)% and 99.0 (± 0.3)% inhibition of MAO-A and MAO-B respectively. In the brains of rats treated with EthC-TCP, MAO-A and MAO-B were inhibited by 79.1 (± 3.4)% and 93.4 (± 0.8)% respectively. These results indicate that the MAO-inhibiting effect observed in vivo is due not to the pro-drug, but to the TCP formed from the pro-drug EthC-TCP.

Since the CE-TCP was observed to be a strong MAO inhibitor (see Table VIII), comparable to TCP under the same conditions, a detailed time-percent inhibition profile of CE-TCP was conducted. Rats, given a dose of CE-TCP (0.1 mmol/kg i.p.) were killed at 5, 15, 30, 60, 120 and 240 min and brains tested for MAO activity. The results are shown in Table X. Both MAO-A and MAO-B were inhibited within 5 min by 55.7 (± 4.0)% and 70.9 (± 5.0)% respectively. Inhibition of MAO-A and MAO-B reached maxima (81.7 ± 5.4 and 84.2 ± 1.3% respectively) 60 min after dosing. Inhibitions of MAO-A and MAO-B were still greater than 80% at 240 min.

A parallel study was conducted with TCP. Rats, injected with TCP (0.1 mmol/kg i.p.), were killed at the same time intervals and brains collected were tested for MAO activity. The time-percent inhibition profile is shown in Table XI. Five min after dosing, MAO-A and MAO-B were inhibited by 70.5 (± 6.8)% and 71.0 (± 4.3)% respectively, and at 15 min inhibition of MAO-A and MAO-B exceeded 80%, at 85.4 (± 4.2)% and 89.5 (± 1.7)% respectively. Inhibition of MAO-A and MAO-B reached maxima (94.0 ± 1.1% and 91.2 ± 1.4%) 30 min after dosing, and both MAO-A and MAO-B remained inhibited by greater than 80% at 240 min. It is interesting to note that the maximum inhibitions of MAO-A and MAO-B were

Table IX. Inhibition of rat brain monoamine oxidase.

	IN VITRO		IN VIVO	
DRUG	% INHIBITION OF MAO-A	% INHIBITION OF MAO-B	% INHIBITION OF MAO-A	% INHIBITION OF MAO-B
EthC-TCP	11.7 (± 4.1)	NIL	79.1 (± 3.4)	93.4 (± 0.8)
TCP	83.9 (± 5.5)	99.0 (± 0.3)	79.1 (± 7.0)	90.8 (± 1.3)

Results represent mean ± S.E.M., N = 6. Concentrations of EthC-TCP and TCP in vitro were 4.8 μM and 9.0 μM respectively, which were equivalent to the concentrations of these compounds found in brain in the in vivo study (as shown in Table V).

Table X. Time-percent inhibition profile of monoamine oxidase in rat brain after administration of N-(2-cyanoethyl)tranylcypromine (CE-TCP) (0.1 mmol/kg i.p.). Values represent mean ± S.E.M. N = 4-6.

TIME (MIN)	% INHIBITION OF MAO-A	% INHIBITION OF MAO-B
5	55.7 (± 4.0)	70.9 (± 5.0)
15	77.5 (± 5.6)	73.4 (± 4.3)
30	78.8 (± 5.4)	75.3 (± 3.0)
60	81.7 (± 5.4)	84.2 (± 1.3)
120	80.4 (± 3.7)	83.0 (± 2.0)
240	81.2 (± 4.9)	80.5 (± 4.0)

Table XI. Time-percent inhibition profile of monoamine oxidase in rat brain after administration of tranylcypromine (TCP) (0.1 mmol/kg i.p.). Values represent mean ± S.E.M. N = 4-6.

TIME (MIN)	% INHIBITION OF MAO-A	% INHIBITION OF MAO-B
5	70.5 (± 6.8)	71.0 (± 4.3)
15	85.4 (± 4.2)	89.5 (± 1.7)
30	94.0 (± 1.1)	91.2 (± 1.4)
60	85.2 (± 4.1)	90.8 (± 1.3)
120	81.5 (± 4.3)	88.0 (± 1.9)
240	80.5 (± 3.9)	84.3 (± 2.6)

at 60 min and 30 min after administration of CE-TCP and TCP respectively, which correlates well with the t_{max} (60 min; time at which concentration is maximum) of CE-TCP as well as TCP formed from CE-TCP in rat brain (Fig. 21) and t_{max} (30 min) of TCP when administered alone (Fig. 20).

C. Brain Levels of β-Phenylethylamine (PEA), Tryptamine (T) and 5-Hydroxytryptamine (5-HT) after Administration of N-(2-Cyanoethyl)tranylcypromine (CE-TCP)

The pro-drug CE-TCP was observed to form TCP in brain after intraperitoneal administration (Fig. 21), and CE-TCP was also found to be a potent MAO inhibitor in its own right (Table VIII). Hence neurochemical studies on CE-TCP were extended and rat brain concentrations of 5-HT, PEA and T were measured at various time intervals after administration of CE-TCP (0.1 mmol/kg i.p.). These biogenic amines have been proposed to be A-specific, B-specific and a mixed substrate respectively for MAO, and were therefore chosen for study. A novel procedure employing aqueous trichloroacetylation was used for the analysis of PEA by ECD-GLC (Baker et al., 1984a) (Section II.E.3). Analysis of T and 5-HT was carried out by a modification of the ECD-GLC procedure of Baker et al. (1980) (see Section II.E.6). The results of this study are shown in Figures 22, 23 and 24. Brain levels of PEA (Fig. 22) showed a steady increase up to 30 min, the levels of PEA being elevated significantly above control values at 5 min ($t = 4.66$, $df = 9$, $p = 0.0006$). There is a marked elevation of PEA levels between 30 and 60 min ($t = 2.31$, $df = 9$, $p = 0.023$), followed by a plateau effect with no significant difference between values at 60 and 240 min ($t = 0.055$, $df = 9$, $p = 0.4786$).

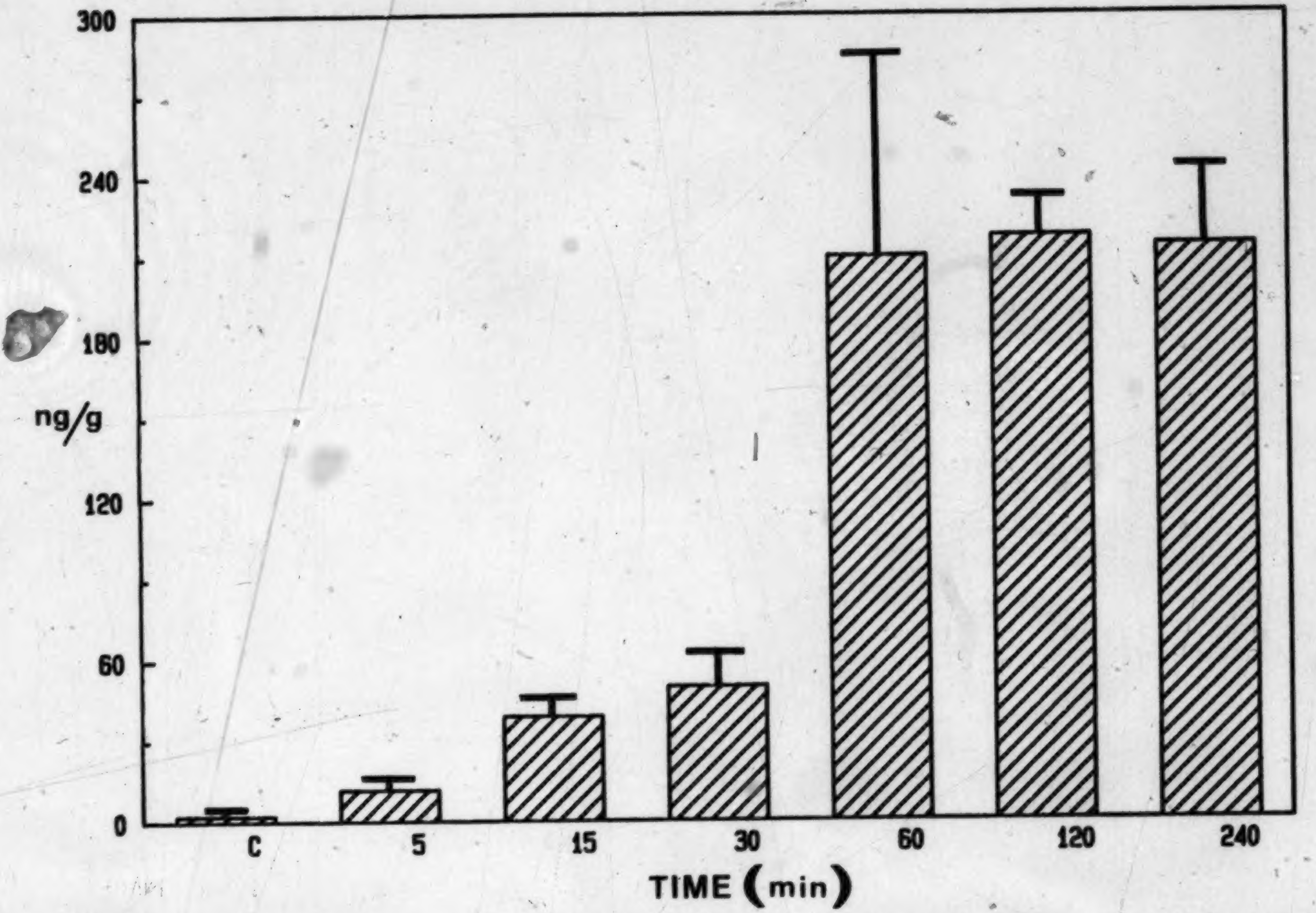


Fig. 22. Brain levels of β-phenylethylamine in the rat after administration of CE-TCP (0.1 mmol/kg i.p.). N = 5-6. Error bars = S.E.M.

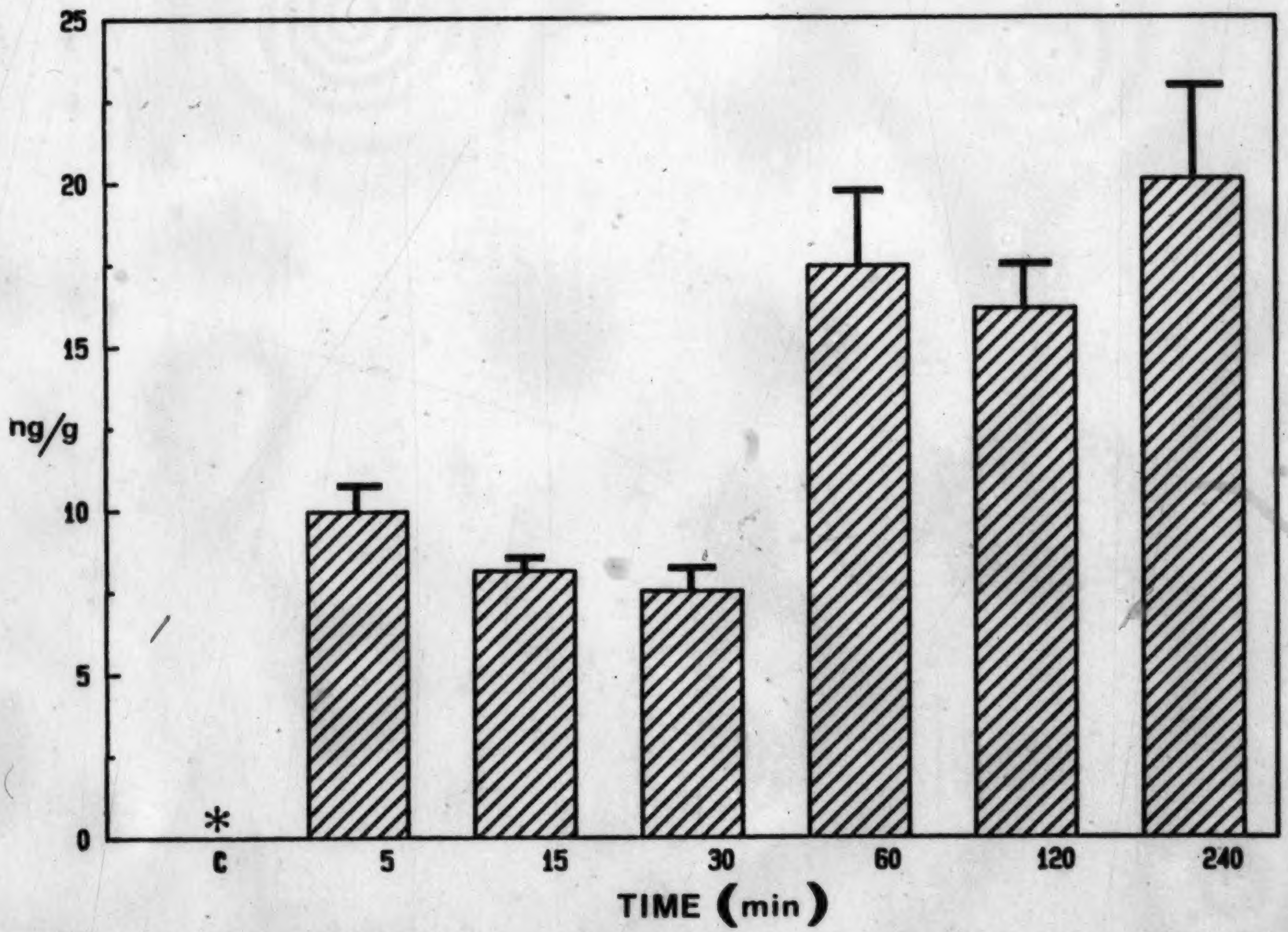


Fig. 23. Brain levels of tryptamine in the rat after administration of CE-TCP (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.
*Control levels of tryptamine are less than 1 ng/g.

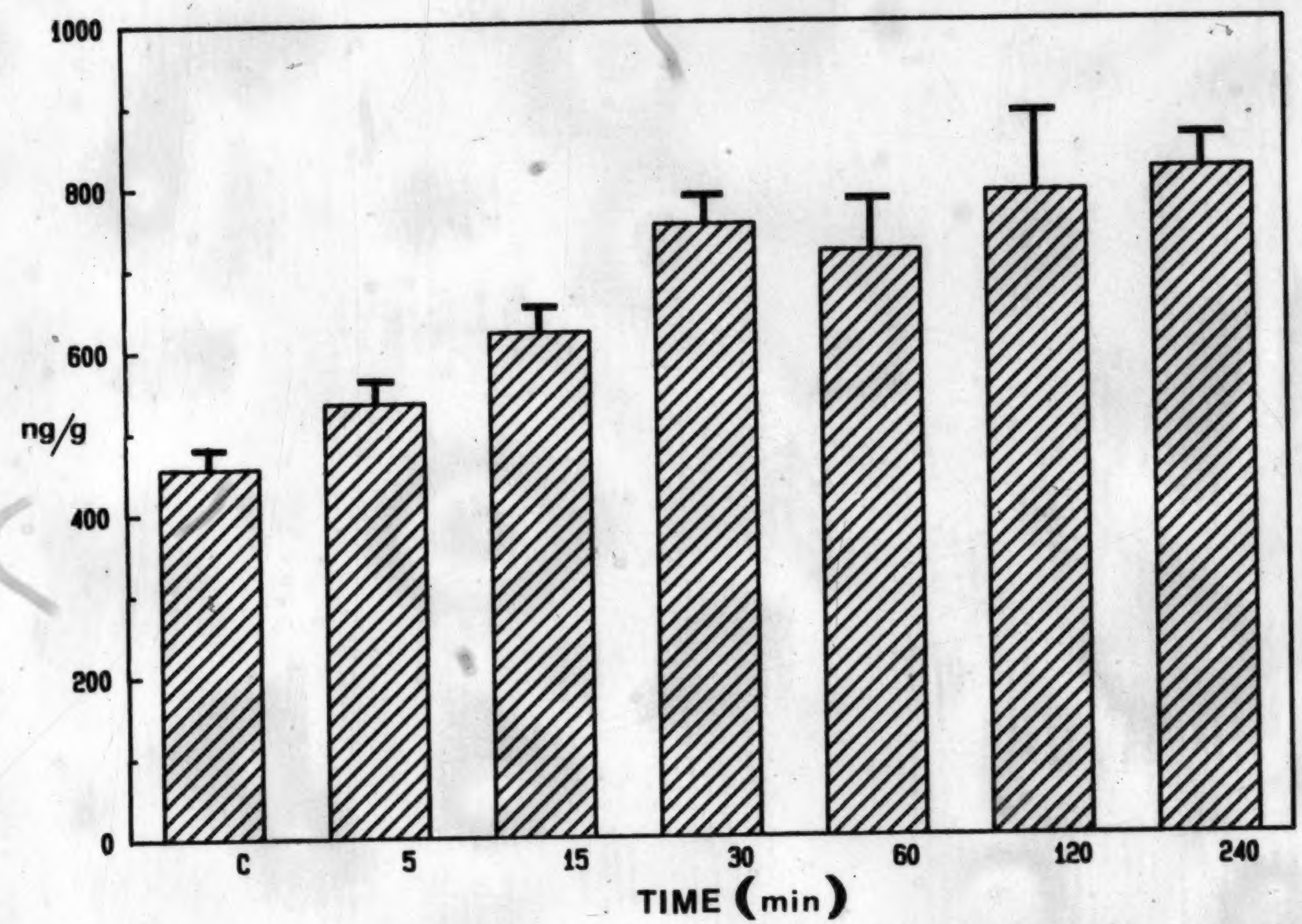


Fig. 24. Brain levels of 5-hydroxytryptamine in the rat after administration of CE-TCP (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.

The concentration of PEA at 240 min (214.1 ± 29 ng/g, mean ± S.E.M.) represents an increase of approximately 100 times control levels ($t = 7.29$, $df = 10$, $p < 0.0001$). Brain levels of T (Fig. 23) also showed an immediate increase after administration of CE-TCP, with levels at 5 min significantly higher than control ($t = 9.7$, $df = 10$, $p < 0.0001$). There was no significant difference between T levels at 5 min and 30 min ($t = 1.79$, $df = 10$, $p > 0.05$); however, the difference between the values at 30 min and 60 min was highly significant ($t = 4.29$, $df = 8$, $p = 0.0013$). Although the levels of T at 240 min were the highest (20.1 ± 2.9 ng/g), representing an increase of approximately 40 times control ($t = 6.85$, $df = 10$, $p < 0.0001$), there was no significant difference between levels at 60 min and 240 min ($t = 0.64$, $df = 8$, $p = 0.2693$) or between 120 min and 240 min ($t = 1.05$, $df = 8$, $p = 0.1607$). Brain 5-HT levels (Fig. 24) at 5 min after administration of CE-TCP were significantly higher than the control value ($t = 2.27$, $df = 8$, $p = 0.0264$). This trend continued with levels at 15 min significantly higher than the levels at 5 min ($t = 1.84$, $df = 10$, $p = 0.0479$), and levels at 30 min significantly higher than levels at 15 min ($t = 2.4$, $df = 10$, $p = 0.0180$). No significant difference in 5-HT levels was observed after 30 min but a general trend indicated the elevation of 5-HT. The brain 5-HT level at 240 min was the highest (818.1 ± 40.2 ng/g), representing an increase of 1.8 times the control value ($t = 6.91$, $df = 8$, $p = 0.0001$).

These results are of interest, particularly since all three of these amines--5-HT, T and PEA--have been implicated in the aetiology of affective disorders (Dewhurst, 1965, 1968; Boulton and Milward, 1971; Van Praag and Korf, 1971; Sabelli and Mosnaim, 1974). It is noteworthy that the increases are much greater with the trace amines PEA and T than with 5-HT. Although TCP has been shown to be formed in brain after administration of CE-TCP (see Fig. 21) (Coutts *et al.*, 1983), the prodrug CE-TCP is a strong MAO inhibitor in its own right and warrants further investigation.

D. Brain Levels of β-Phenylethylamine (PEA), Tryptamine (T) and 5-Hydroxytryptamine (5-HT) after Administration of Tranylcypromine (TCP)

A parallel study was conducted where an equimolar dose of TCP (0.1 mmol/kg i.p.) was administered to rats and brain levels of PEA, T and 5-HT were measured at various time intervals. Analysis of PEA was performed by the ECD-GLC procedure of Hampson *et al.* (1984a) (Section II.E.2), and 5-HT and T were measured by a modification of the ECD-GLC procedure of Baker *et al.* (1980) (Section II.E.6). The results of the study are shown in Figures 25, 26 and 27. Brain levels of PEA (Fig. 25) were elevated rapidly, reaching a concentration of 84.2 (± 6.3) ng/g by 5 min ($t = 13.09$, df = 10, $p < 0.0001$). Brain PEA levels continued to rise with levels at 60 min significantly higher than the levels at 30 min ($t = 2.71$, df = 10, $p = 0.0110$). The PEA levels reached a maximum (191.8 ± 20.1 ng/g) at 120 min, which is an increase of approximately 90 times the control value ($t = 9.43$, df = 10, $p < 0.0001$). At 240 min the brain PEA levels had fallen to 91.6 (± 16.4) ng/g but were still significantly higher than the control value ($t = 5.46$, df = 10, $p = 0.0001$). Brain levels of T (Fig. 26) increase rapidly after administration of TCP. At 5 min the levels of T are significantly higher than the control value ($t = 6.12$, df = 9, $p = 0.0001$), as were T levels at all subsequent time periods. Brain T levels between successive time intervals (after

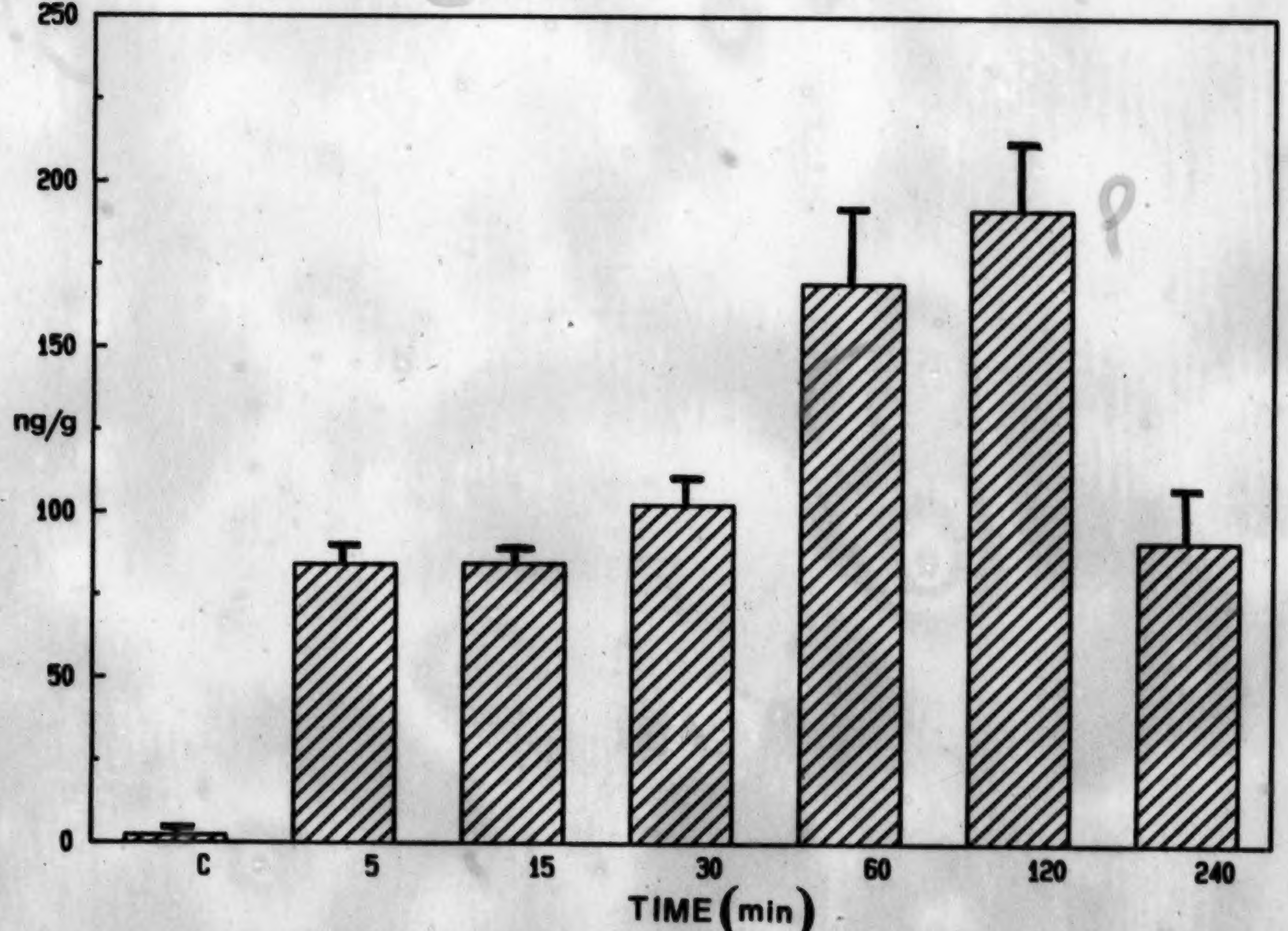


Fig. 25. Brain levels of β-phenylethylamine in the rat after administration of TCP (0.1 mmol/kg i.p.). N = 6. Error bars = S.E.M.

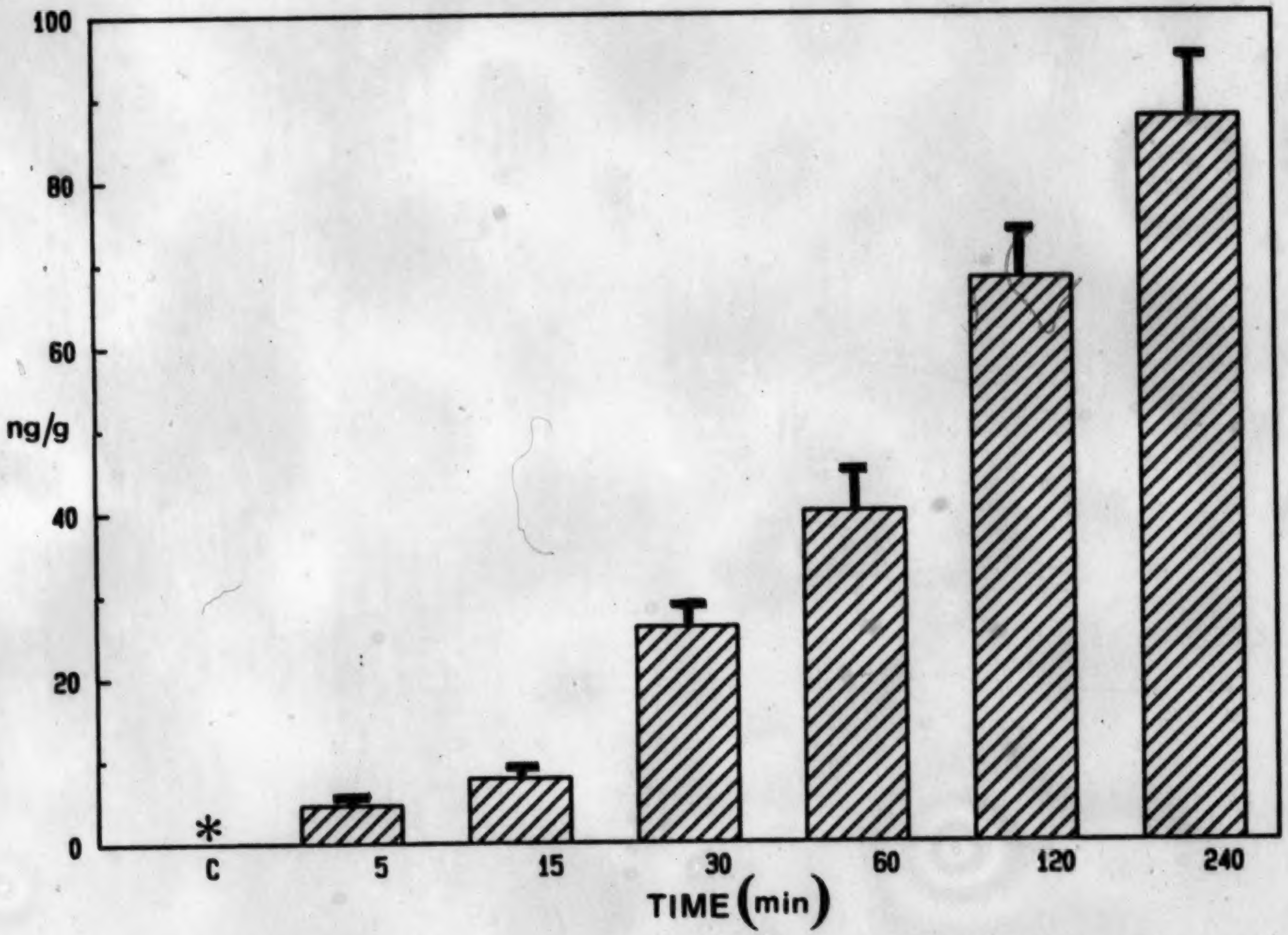


Fig. 26. Brain levels of tryptamine in the rat after administration of TCP (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.

*Control brain levels of tryptamine are less than 1 ng/g.

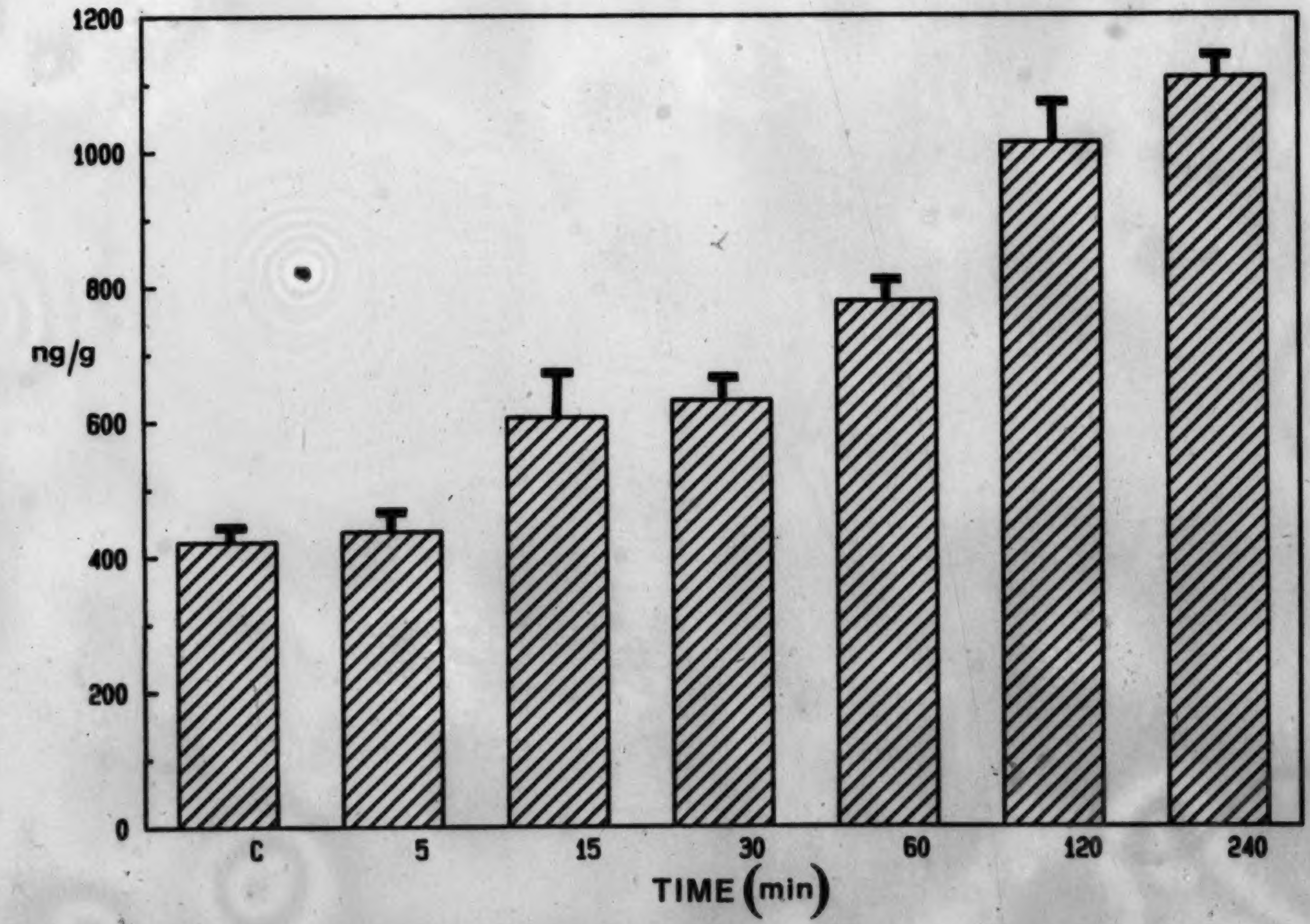


Fig. 27. Brain levels of 5-hydroxytryptamine in the rat after administration of TCP (0.1 mmol/kg i.p.). N = 4-6. Error bars = S.E.M.

15 min) were significantly elevated from the time intervals immediately preceding. At 240 min T levels remained at a high of 87.5 ± 7.9 ng/g, representing an increase of approximately 175 times the control value ($t = 19.59$, $df = 8$, $p < 0.0001$), assuming a control level of 0.5 ng/g as found by Philips _et al._ (1974) using high resolution mass spectrometry. Philips and Boulton (1979) reported similar findings in rat striatum using a 10 mg/kg dose of TCP. They noted that PEA and T reached peak levels at 120 min and 240 min respectively after i.p. administration. Brain levels of 5-HT (Fig. 27) also showed gradual elevation. At 60 min the levels of 5-HT were significantly higher than the levels at 30 min ($t = 3.33$, $df = 10$, $p = 0.0038$) and levels at 120 min were significantly higher than the levels at 60 min ($t = 3.32$, $df = 10$, $p = 0.0038$). Brain levels of 5-HT remained highest at 240 min, with levels reaching 1108.3 (± 34.3) ng/g. This value represents an increase of approximately 2.6 times the control value ($t = 13.66$, $df = 8$, $p < 0.0001$).

Comparing these results with those of CE-TCP, it can be observed that both TCP and CE-TCP elevate the levels of all three amines in brain and in particular the trace amines PEA and T. The levels of PEA remained the highest at 120 min after administration of CE-TCP and TCP, representing an increase of approximately 100 and 90 times the control values respectively. However, levels of PEA dropped (91.6 ± 16.4 ng/g) at 240 min after administration of TCP but remained high (214.1 ± 29 ng/g) at this time after administration of CE-TCP. It may be that since CE-TCP is an MAO inhibitor in its own right (Table VIII), the concentrations of CE-TCP and the TCP formed from it may together have a synergistic effect on MAO activity in brain. This may be one explanation for the elevated level of PEA at 240 min after administration of the pro-drug CE-TCP. The brain levels of T were elevated to approximately 40 and 175 times control value after CE-TCP and TCP respectively, and brain levels of 5-HT were elevated by approximately 1.8 and 2.6 times control values after CE-TCP and TCP respectively.

These results indicate that both CE-TCP and TCP elevate the levels of trace amines PEA and T to a much greater extent above control values than they elevate the levels of 5-HT. However, the pro-drug CE-TCP caused a greater elevation of PEA levels than did an equimolar dose of TCP. In contrast, TCP caused higher levels of T and 5-HT than those observed after an equimolar dose of CE-TCP. This study indicates that future investigations of such pro-drugs should involve studies of their effects on trace amines in addition to the more classical amines such as 5-HT.

E. Mass Spectra of the Derivatives

Structures of the final derivatives synthesized for ECD-GLC analysis of the bioactive amines and analogues were confirmed by gas-liquid chromatography-mass spectrometry (GLC-MS). The mass spectral fragmentation patterns of the derivatives are illustrated in Figures 28 to 45.

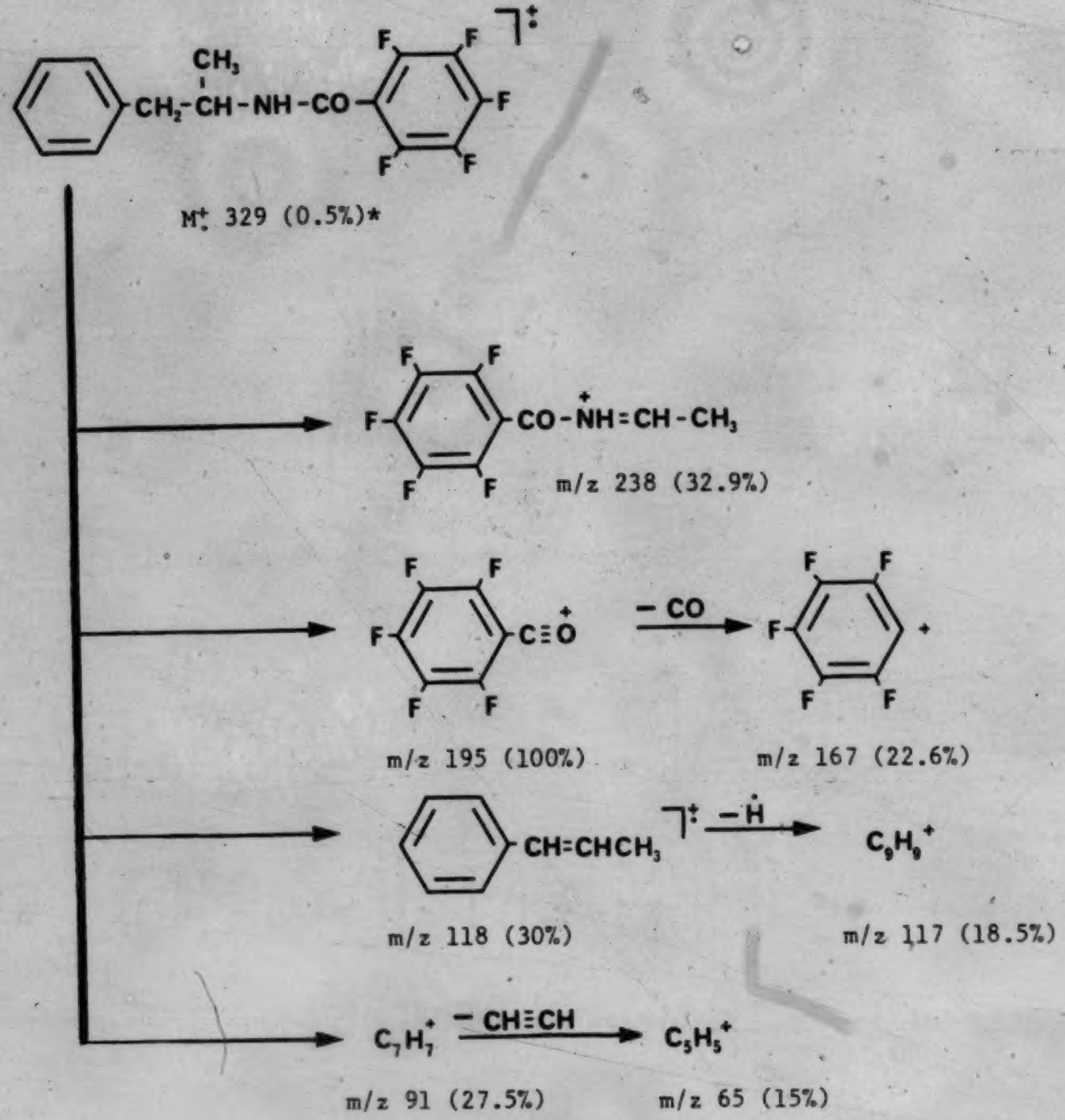
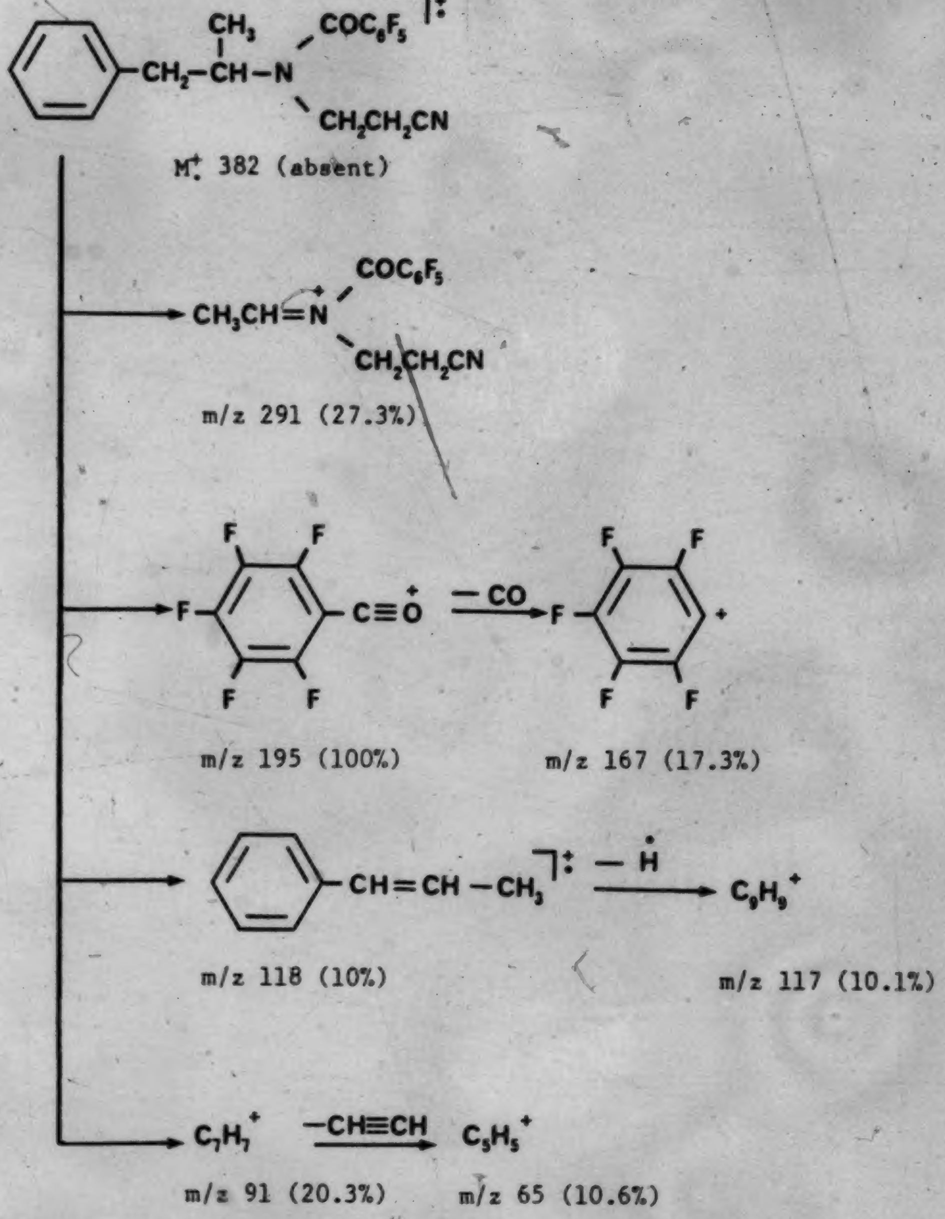

CH₃
CH₂-CH-NH-CO
F
M⁺ 329 (0.5%)*
CO-NH=CH-CH₃
m/z 238 (32.9%)
C≡O
− CO
m/z 195 (100%)
m/z 167 (22.6%)
CH=CHCH₃
− H
C₉H₉⁺
m/z 118 (30%)
m/z 117 (18.5%)
C₇H₇⁺
− CH≡CH
C₅H₅⁺
m/z 91 (27.5%)
m/z 65 (15%)

Fig. 28. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of amphetamine. *Numbers in parentheses are percent relative abundance of the individual fragments.



M⁺˙ 382 (absent)
m/z 291 (27.3%)
m/z 195 (100%)
− CO
m/z 167 (17.3%)
m/z 118 (10%)
− H˙
m/z 117 (10.1%)
−CH≡CH
m/z 91 (20.3%)
m/z 65 (10.6%)

Fig. 29. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of N-(2-cyanoethyl)amphetamine.

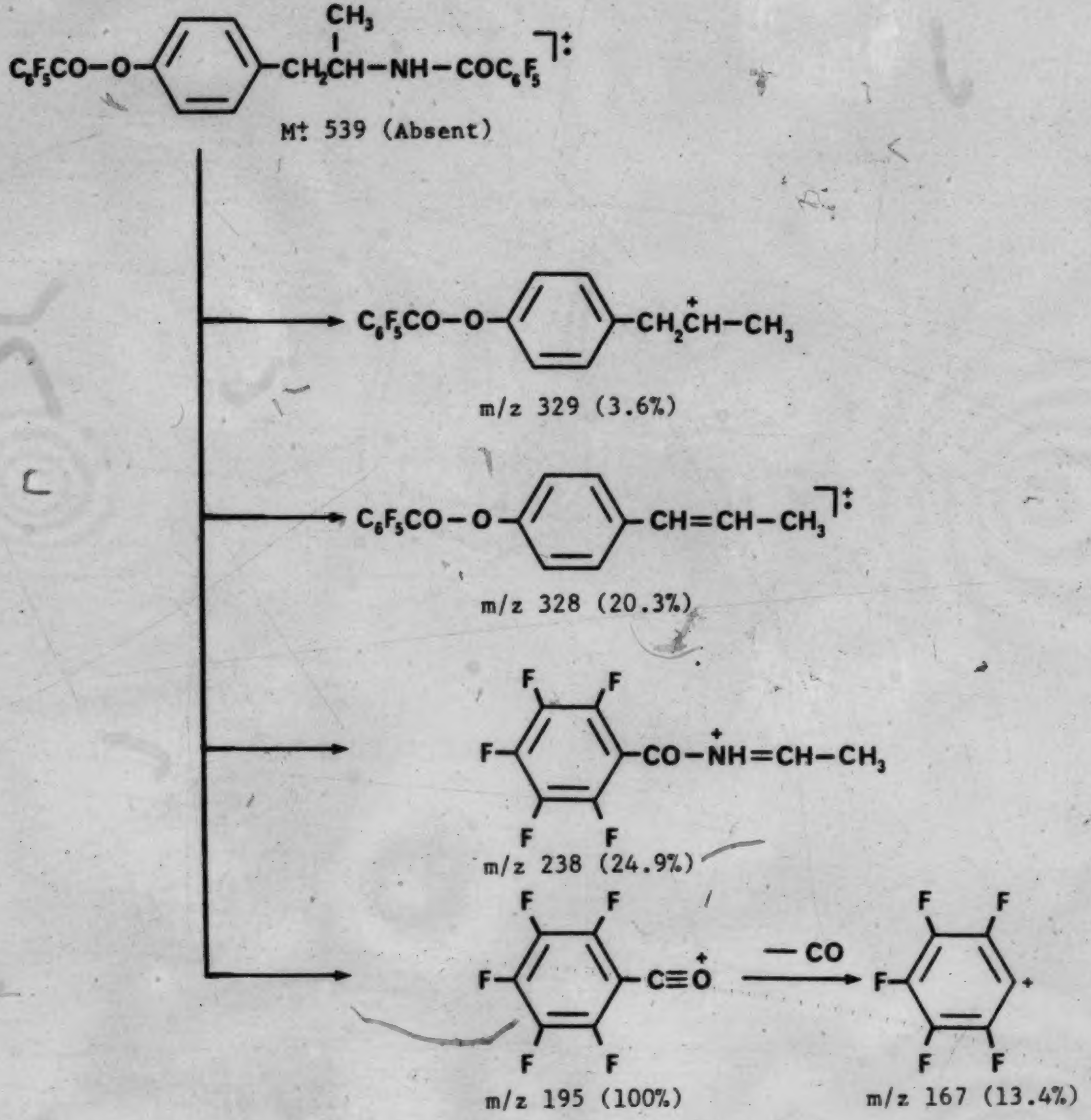

C6F5CO–O–C6H4–CH2CH(CH3)–NH–COC6F5]+·
M+· 539 (Absent)
C6F5CO–O–C6H4–CH2C+H–CH3
m/z 329 (3.6%)
C6F5CO–O–C6H4–CH=CH–CH3]+·
m/z 328 (20.3%)
C6F5–CO–N+H=CH–CH3
m/z 238 (24.9%)
C6F5–C≡O+
m/z 195 (100%)
— CO
C6F5+
m/z 167 (13.4%)

Fig. 30. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of _para_-hydroxyamphetamine.

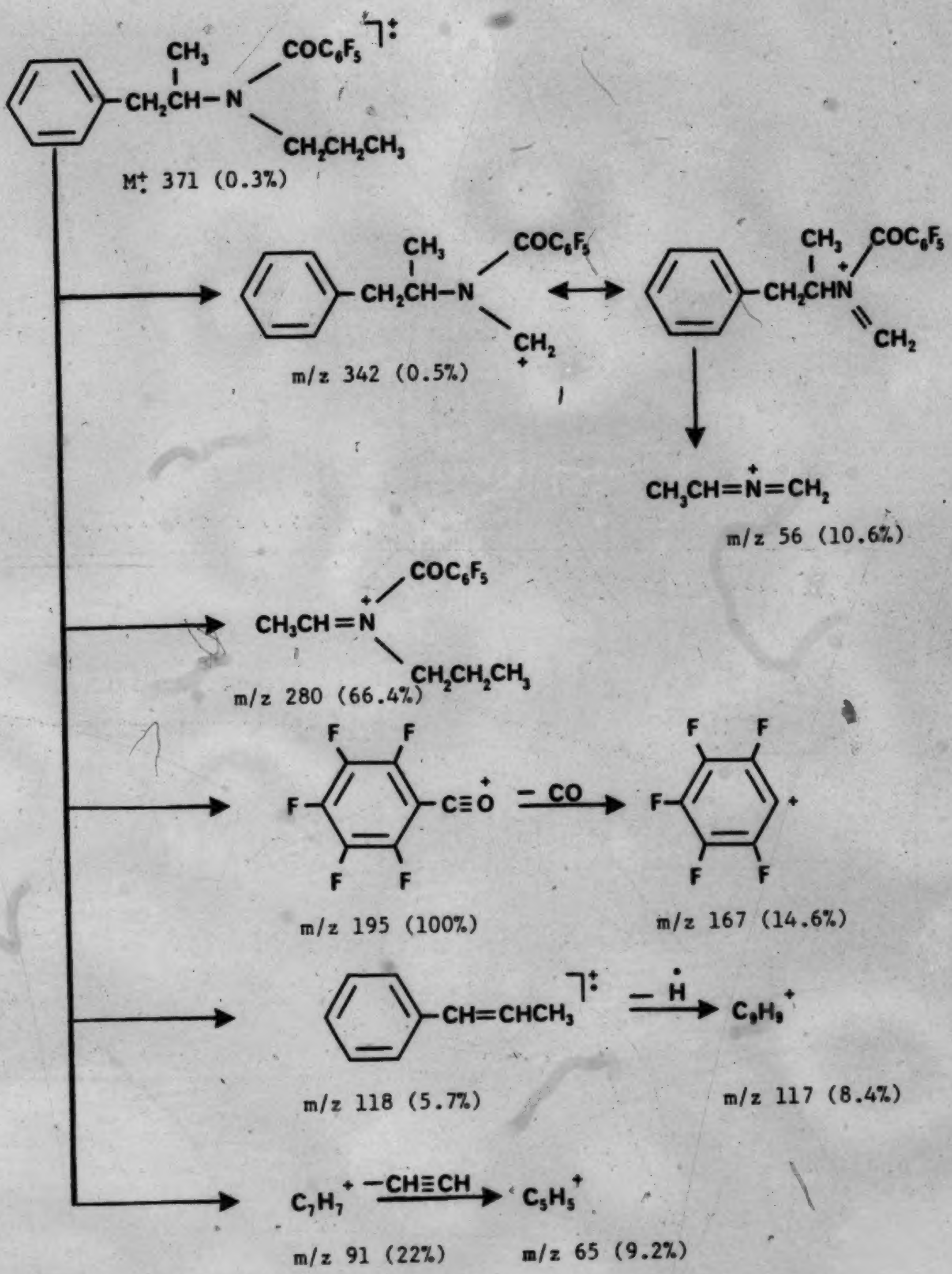

M⁺· 371 (0.3%)
m/z 342 (0.5%)
m/z 56 (10.6%)
m/z 280 (66.4%)
m/z 195 (100%)
− CO
m/z 167 (14.6%)
− H·
m/z 118 (5.7%)
m/z 117 (8.4%)
−CH≡CH
m/z 91 (22%)
m/z 65 (9.2%)

Fig. 31. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of N-n-propylamphetamine.

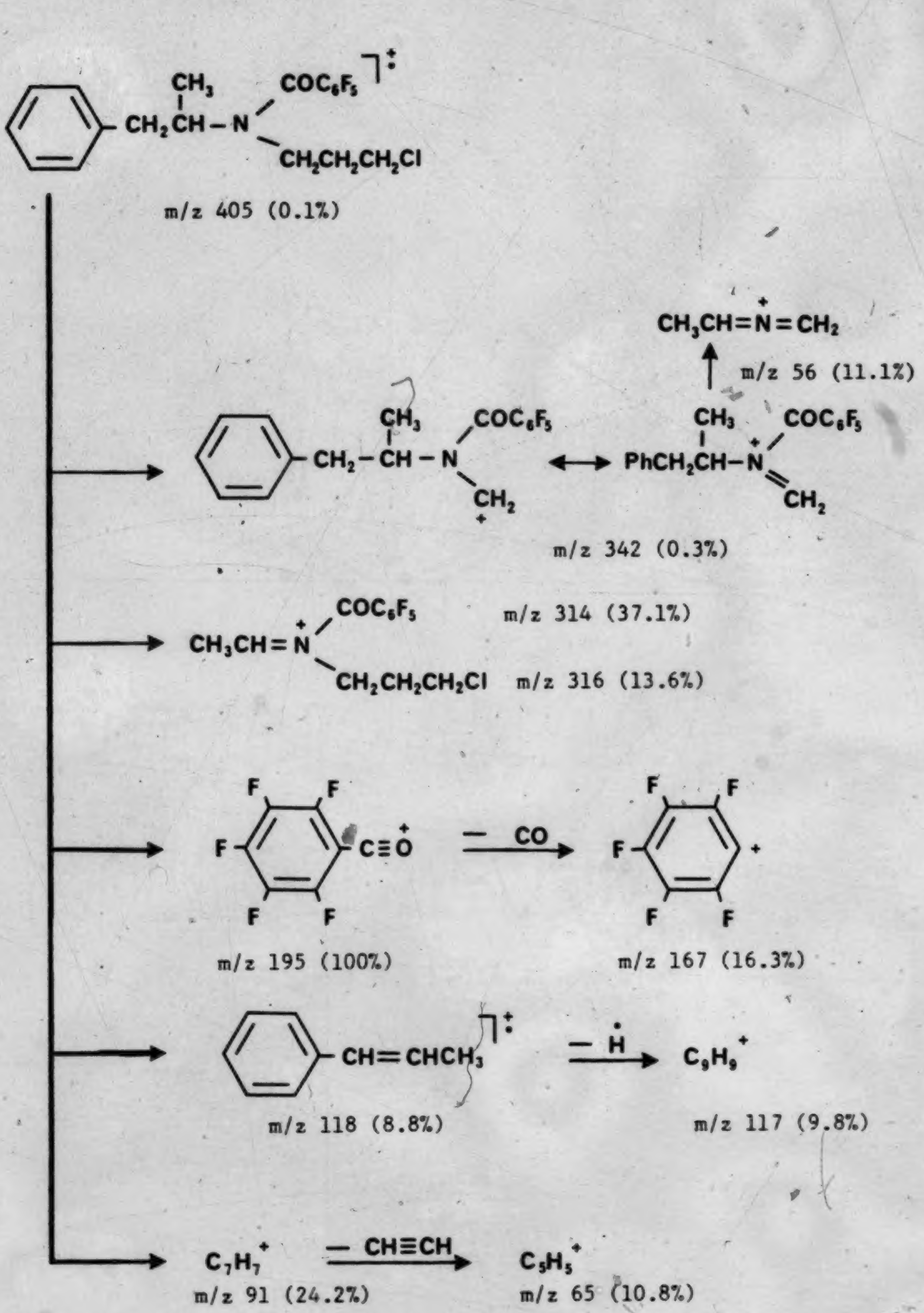

m/z 405 (0.1%)
m/z 56 (11.1%)
m/z 342 (0.3%)
m/z 314 (37.1%)
m/z 316 (13.6%)
− CO
m/z 195 (100%)
m/z 167 (16.3%)
m/z 118 (8.8%)
C₉H₉⁺
m/z 117 (9.8%)
C₇H₇⁺
− CH≡CH
C₅H₅⁺
m/z 91 (24.2%)
m/z 65 (10.8%)

Fig. 32. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of N-(3-chloropropyl)amphetamine.

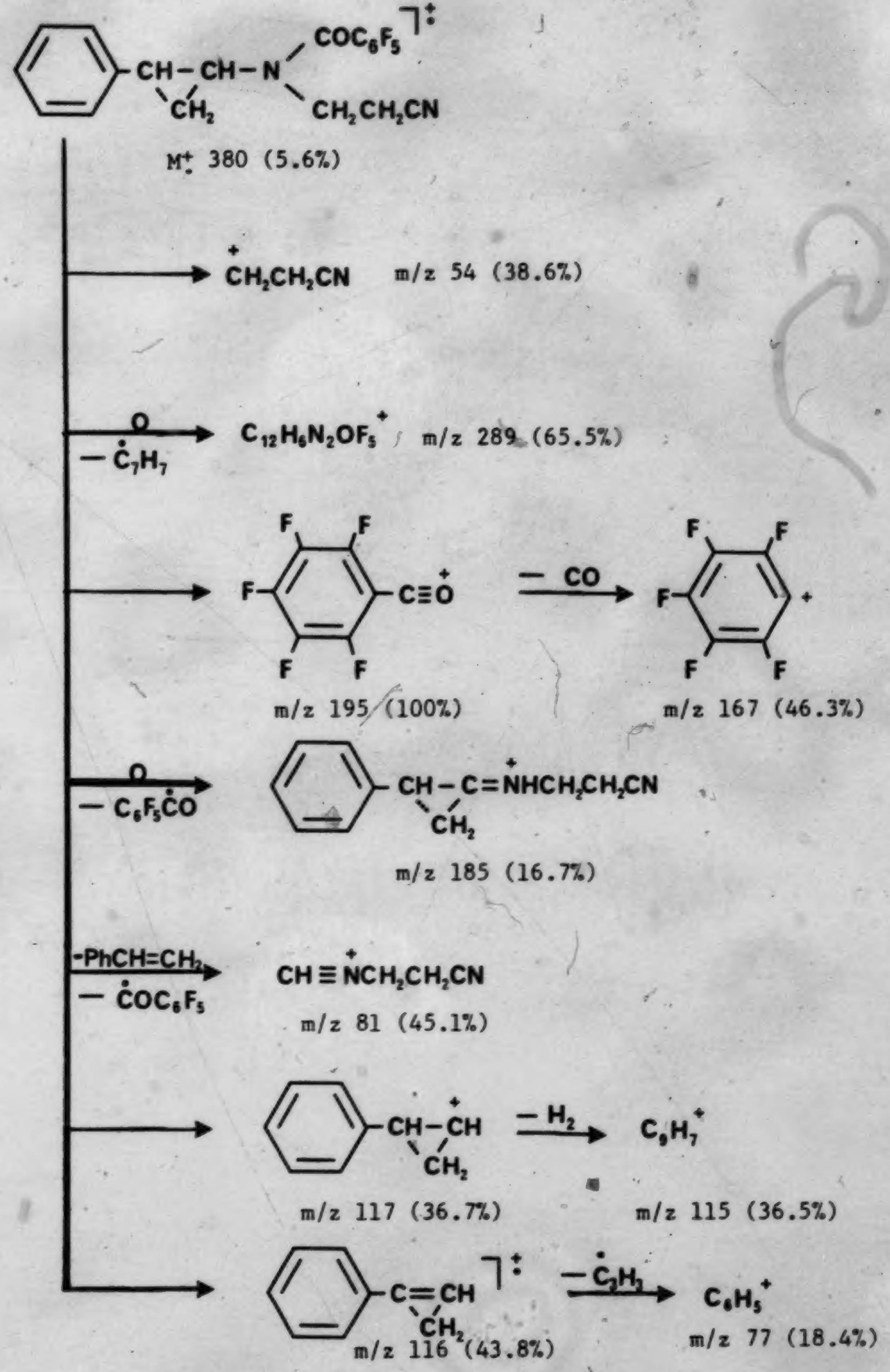

COC₆F₅
CH–CH–N
CH₂
CH₂CH₂CN
M⁺· 380 (5.6%)
CH₂CH₂CN
m/z 54 (38.6%)
O
– C₇H₇
C₁₂H₆N₂OF₅⁺
m/z 289 (65.5%)
F
C≡O
– CO
m/z 195 (100%)
m/z 167 (46.3%)
O
– C₆F₅CO
CH – C=NHCH₂CH₂CN
CH₂
m/z 185 (16.7%)
-PhCH=CH₂
– COC₆F₅
CH≡NCH₂CH₂CN
m/z 81 (45.1%)
CH–CH
CH₂
– H₂
C₉H₇⁺
m/z 117 (36.7%)
m/z 115 (36.5%)
C=CH
CH₂
– C₃H₃
C₆H₅⁺
m/z 116 (43.8%)
m/z 77 (18.4%)

Fig. 33. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of N-(2-cyanoethyl)tranylcypromine.

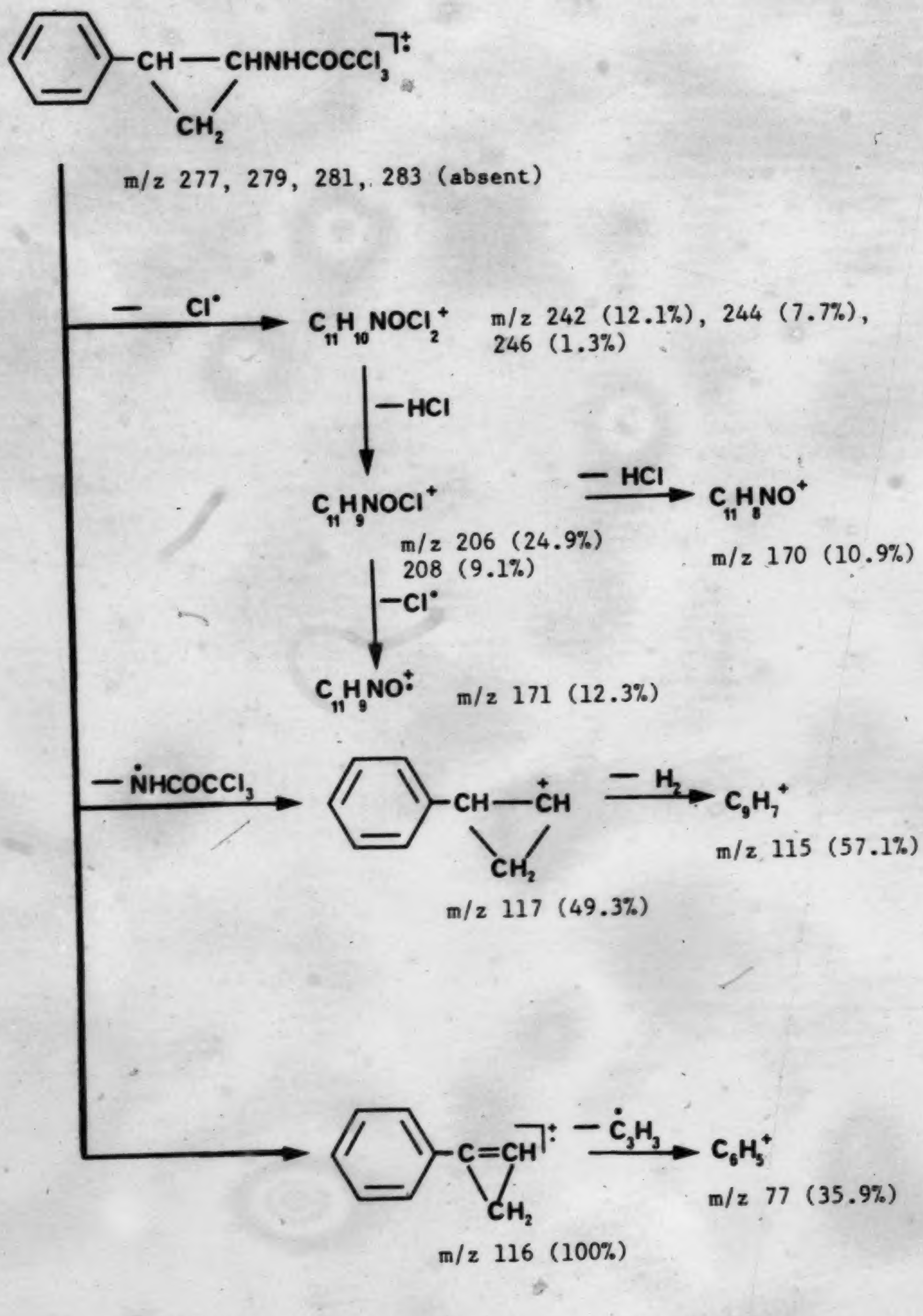

CH — CHNHCOCCl3]+·
CH2
m/z 277, 279, 281, 283 (absent)
— Cl•
C11H10NOCl2+
m/z 242 (12.1%), 244 (7.7%), 246 (1.3%)
—HCl
C11H9NOCl+
m/z 206 (24.9%) 208 (9.1%)
— HCl
C11H8NO+
m/z 170 (10.9%)
—Cl•
C11H9NO+·
m/z 171 (12.3%)
— •NHCOCCl3
CH — +CH
CH2
m/z 117 (49.3%)
— H2
C9H7+
m/z 115 (57.1%)
C=CH]+·
CH2
m/z 116 (100%)
— •C3H3
C6H5+
m/z 77 (35.9%)

Fig. 34. Probable mass spectral fragmentation of the trichloroacetyl derivative of tranylcypromine.

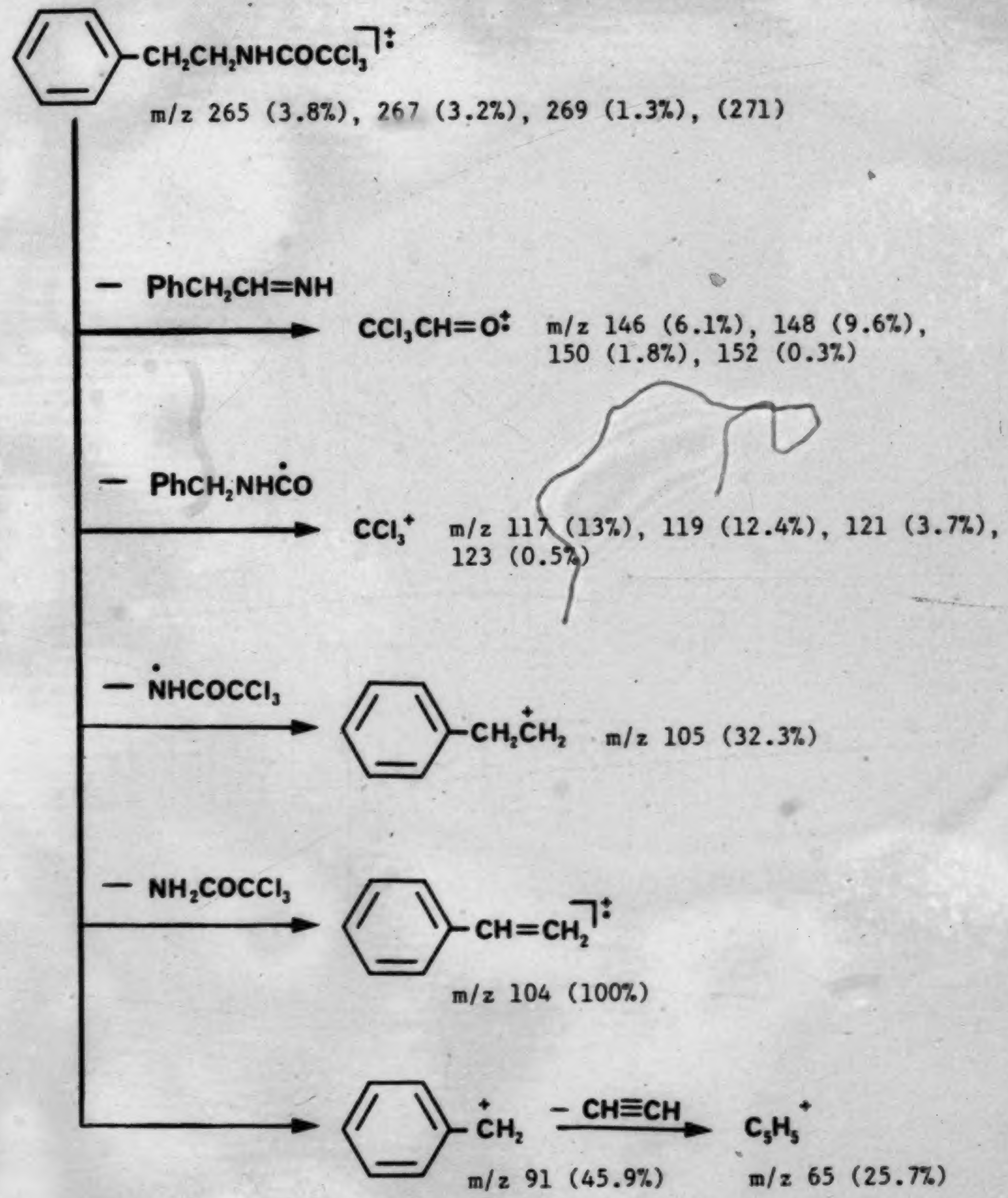

CH₂CH₂NHCOCCl₃ ⁺·
m/z 265 (3.8%), 267 (3.2%), 269 (1.3%), (271)
− PhCH₂CH=NH
CCl₃CH=O⁺·
m/z 146 (6.1%), 148 (9.6%), 150 (1.8%), 152 (0.3%)
− PhCH₂NHĊO
CCl₃⁺
m/z 117 (13%), 119 (12.4%), 121 (3.7%), 123 (0.5%)
− ṄHCOCCl₃
CH₂ĊH₂
m/z 105 (32.3%)
− NH₂COCCl₃
CH=CH₂ ⁺·
m/z 104 (100%)
ĊH₂
− CH≡CH
C₅H₅⁺
m/z 91 (45.9%)
m/z 65 (25.7%)

Fig. 35. Probable mass spectral fragmentation of the trichloroacetyl derivative of β-phenylethylamine.

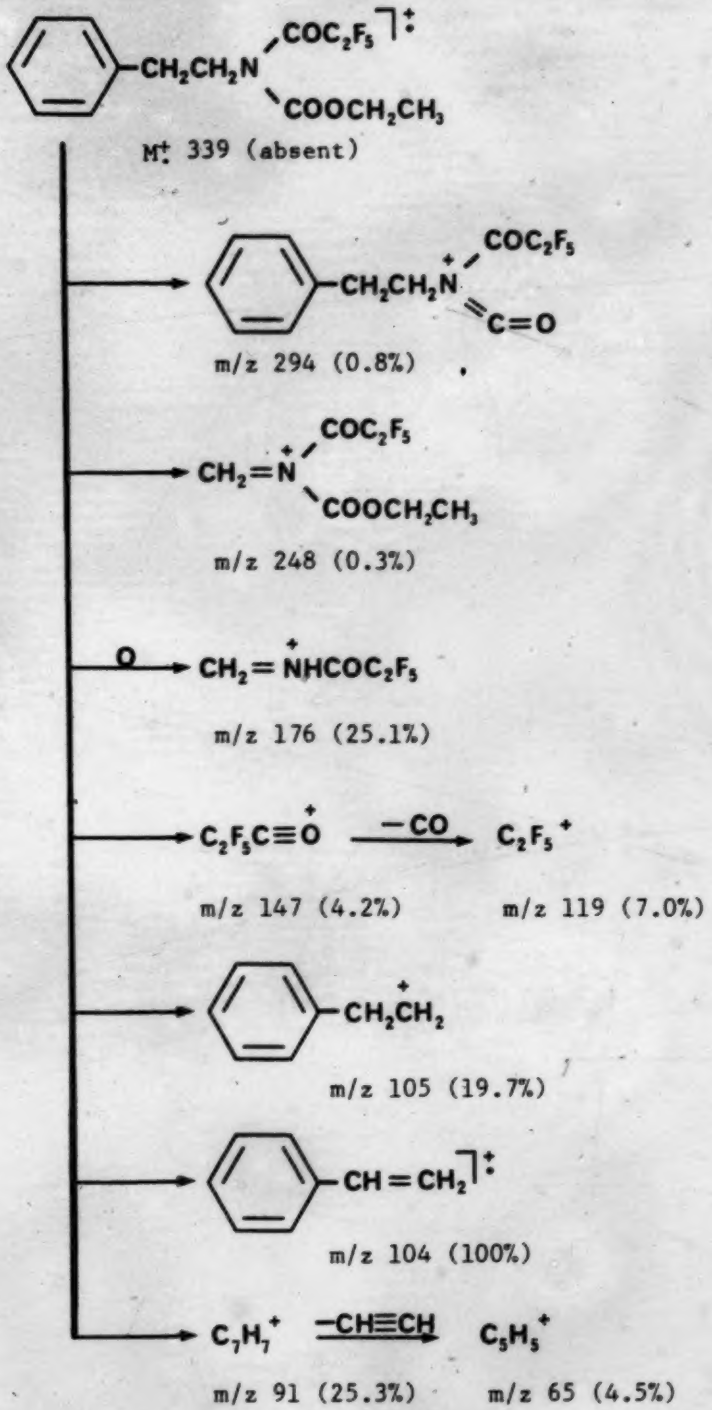

M⁺· 339 (absent)
m/z 294 (0.8%)
m/z 248 (0.3%)
CH₂=N⁺HCOC₂F₅
m/z 176 (25.1%)
C₂F₅C≡O⁺
−CO
C₂F₅⁺
m/z 147 (4.2%)
m/z 119 (7.0%)
m/z 105 (19.7%)
m/z 104 (100%)
C₇H₇⁺
−CH≡CH
C₅H₅⁺
m/z 91 (25.3%)
m/z 65 (4.5%)

Fig. 36. Probable mass spectral fragmentation of pentafluoropropionyl derivative of N-(ethoxycarbonyl)phenylethylamine.

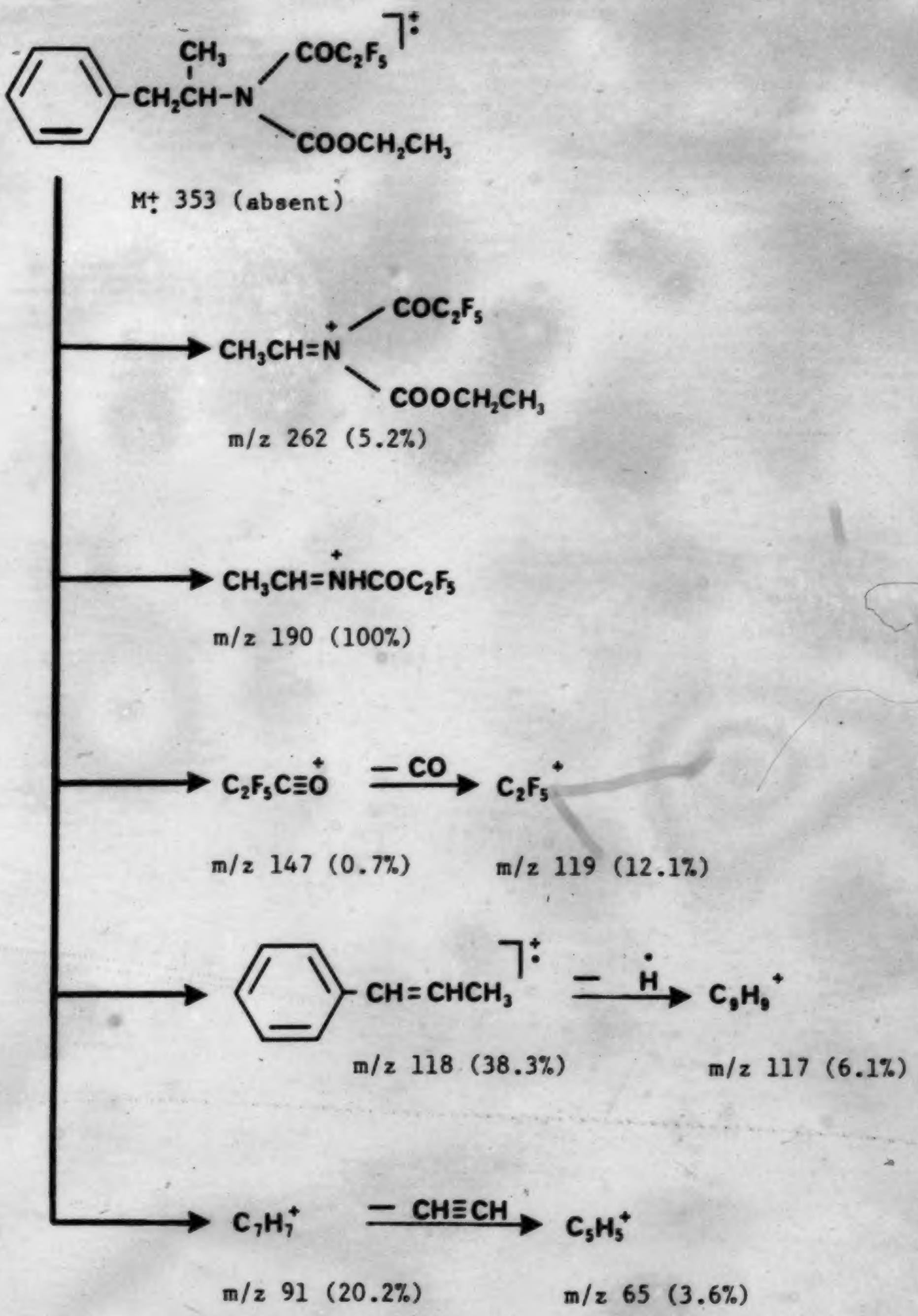

CH₃
CH₂CH-N
COC₂F₅
COOCH₂CH₃
M+̣ 353 (absent)
CH₃CH=N⁺
COC₂F₅
COOCH₂CH₃
m/z 262 (5.2%)
CH₃CH=N⁺HCOC₂F₅
m/z 190 (100%)
C₂F₅C≡O⁺
— CO
C₂F₅⁺
m/z 147 (0.7%)
m/z 119 (12.1%)
CH=CHCH₃
— H
C₉H₉⁺
m/z 118 (38.3%)
m/z 117 (6.1%)
C₇H₇⁺
— CH≡CH
C₅H₅⁺
m/z 91 (20.2%)
m/z 65 (3.6%)

Fig. 37. Probable mass spectral fragmentation of pentafluoropropionyl derivative of N-(ethoxycarbonyl)amphetamine.

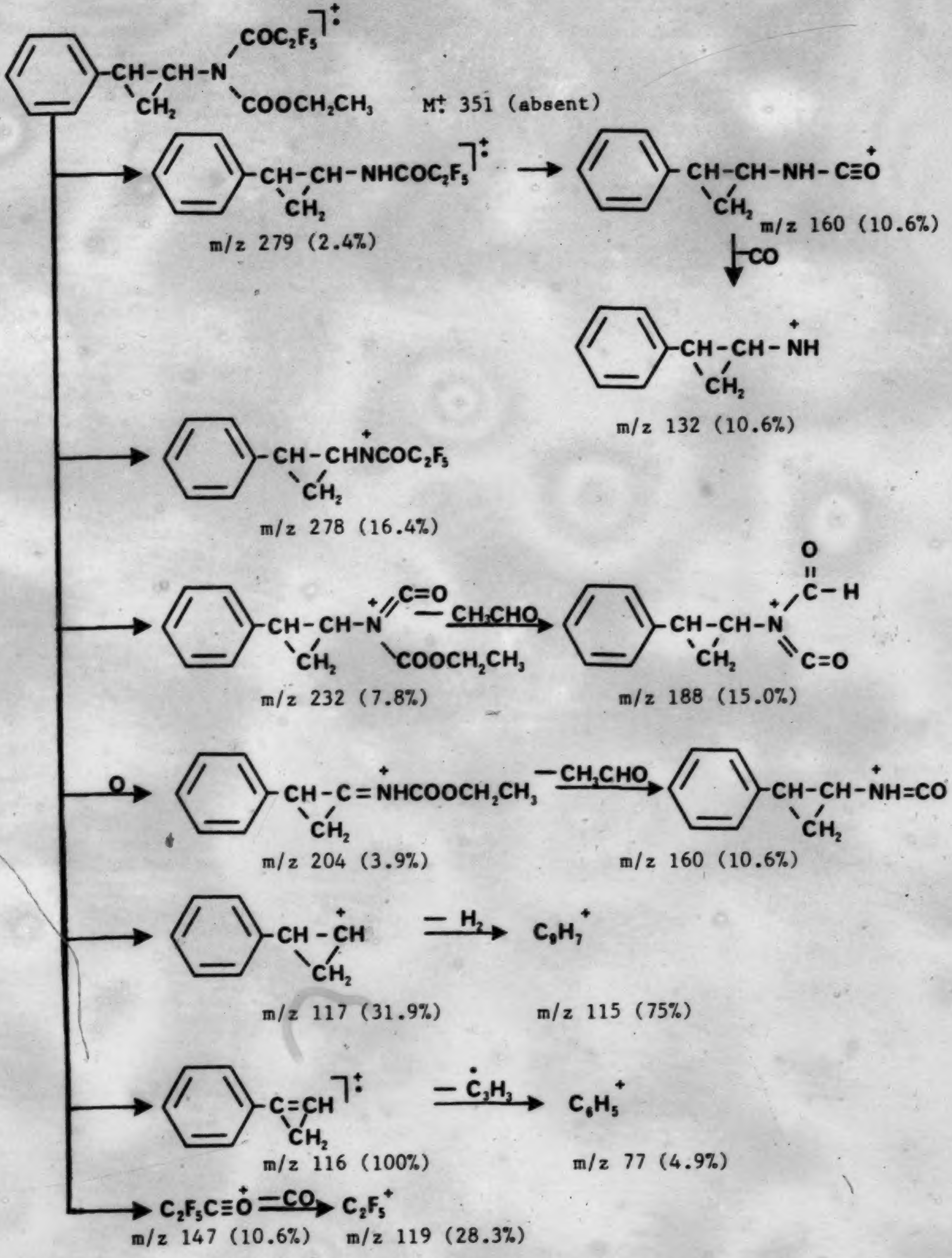

M⁺ 351 (absent)
m/z 279 (2.4%)
m/z 160 (10.6%)
−CO
m/z 132 (10.6%)
m/z 278 (16.4%)
−CH₃CHO
m/z 232 (7.8%)
m/z 188 (15.0%)
O
−CH₃CHO
m/z 204 (3.9%)
m/z 160 (10.6%)
−H₂
C₉H₇⁺
m/z 117 (31.9%)
m/z 115 (75%)
−C₃H₃
C₆H₅⁺
m/z 116 (100%)
m/z 77 (4.9%)
C₂F₅C≡O⁺
−CO
C₂F₅⁺
m/z 147 (10.6%)
m/z 119 (28.3%)

Fig. 38. Probable mass spectral fragmentation of pentafluoropropionyl derivative of N-(ethoxycarbonyl)tranylcypromine.

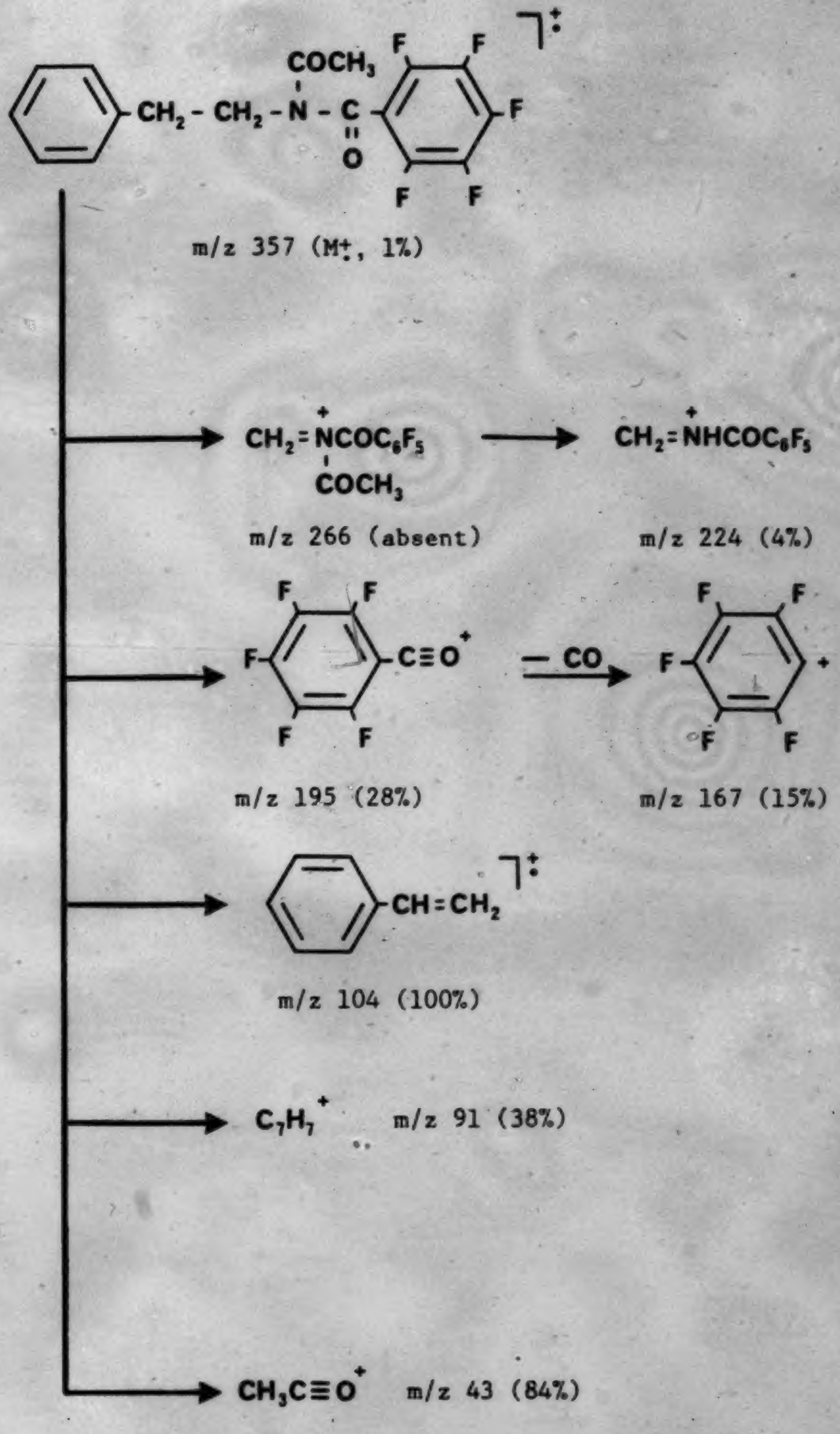

COCH₃
CH₂ - CH₂ - N - C
O
F F F F F
m/z 357 (M⁺·, 1%)
CH₂=N⁺COC₆F₅
COCH₃
m/z 266 (absent)
CH₂=N⁺HCOC₆F₅
m/z 224 (4%)
C≡O⁺
m/z 195 (28%)
− CO
m/z 167 (15%)
CH=CH₂
m/z 104 (100%)
C₇H₇⁺
m/z 91 (38%)
CH₃C≡O⁺
m/z 43 (84%)

Fig. 39. Probable mass spectral fragmentation of derivatized β-phenylethylamine (acetylation followed by pentafluorobenzoylation).

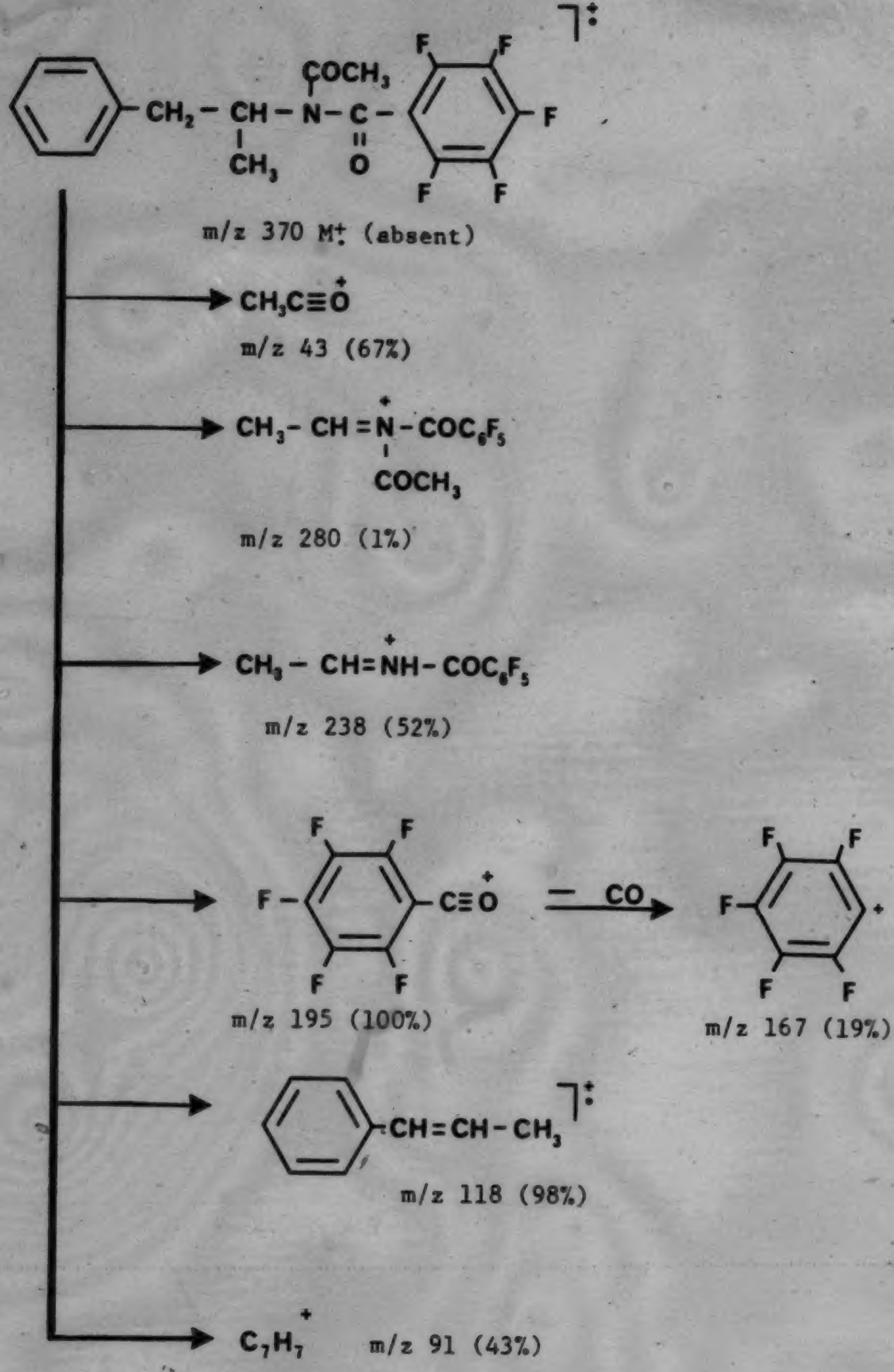

m/z 370 M+· (absent)
CH3C≡O+
m/z 43 (67%)
CH3-CH=N+-COC6F5
COCH3
m/z 280 (1%)
CH3-CH=N+H-COC6F5
m/z 238 (52%)
C≡O+
-CO
m/z 195 (100%)
m/z 167 (19%)
CH=CH-CH3
m/z 118 (98%)
C7H7+
m/z 91 (43%)

Fig. 40. Probable mass spectral fragmentation of derivatized amphetamine (acetylation followed by pentafluorobenzoylation).

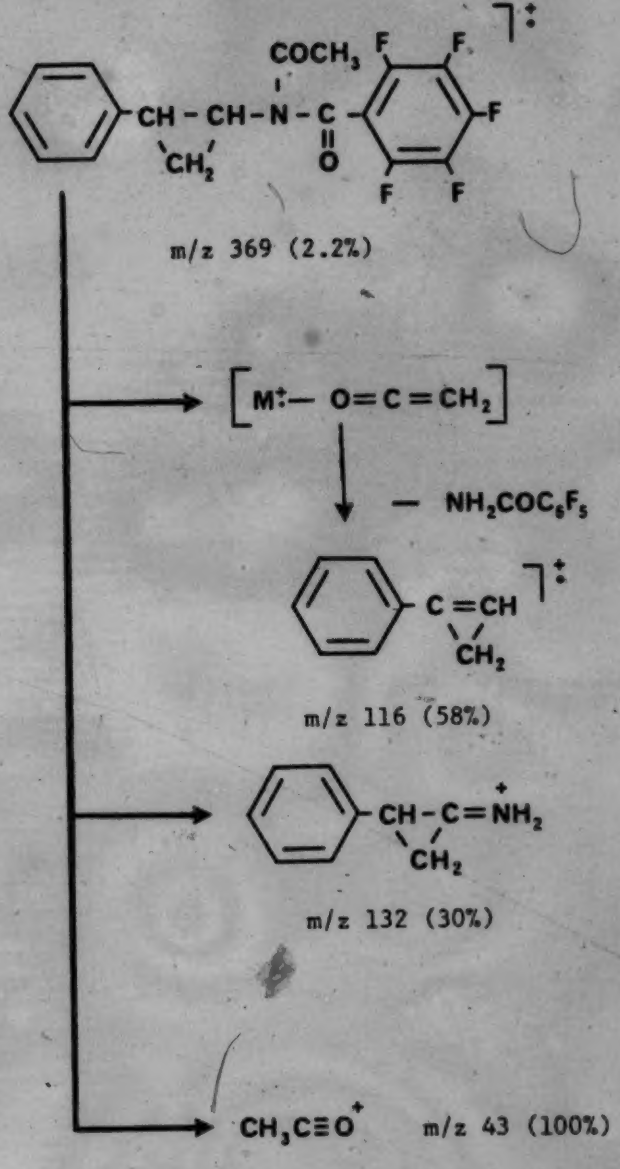

COCH₃
F
F
F
F
F
CH - CH - N - C
O
CH₂
m/z 369 (2.2%)
[M⁺· − O=C=CH₂]
− NH₂COC₆F₅
C = CH
CH₂
m/z 116 (58%)
CH - C = N⁺H₂
CH₂
m/z 132 (30%)
CH₃C≡O⁺
m/z 43 (100%)

Fig. 41. Probable mass spectral fragmentation of derivatized tranylcypromine (acetylation followed by pentafluorobenzoylation).

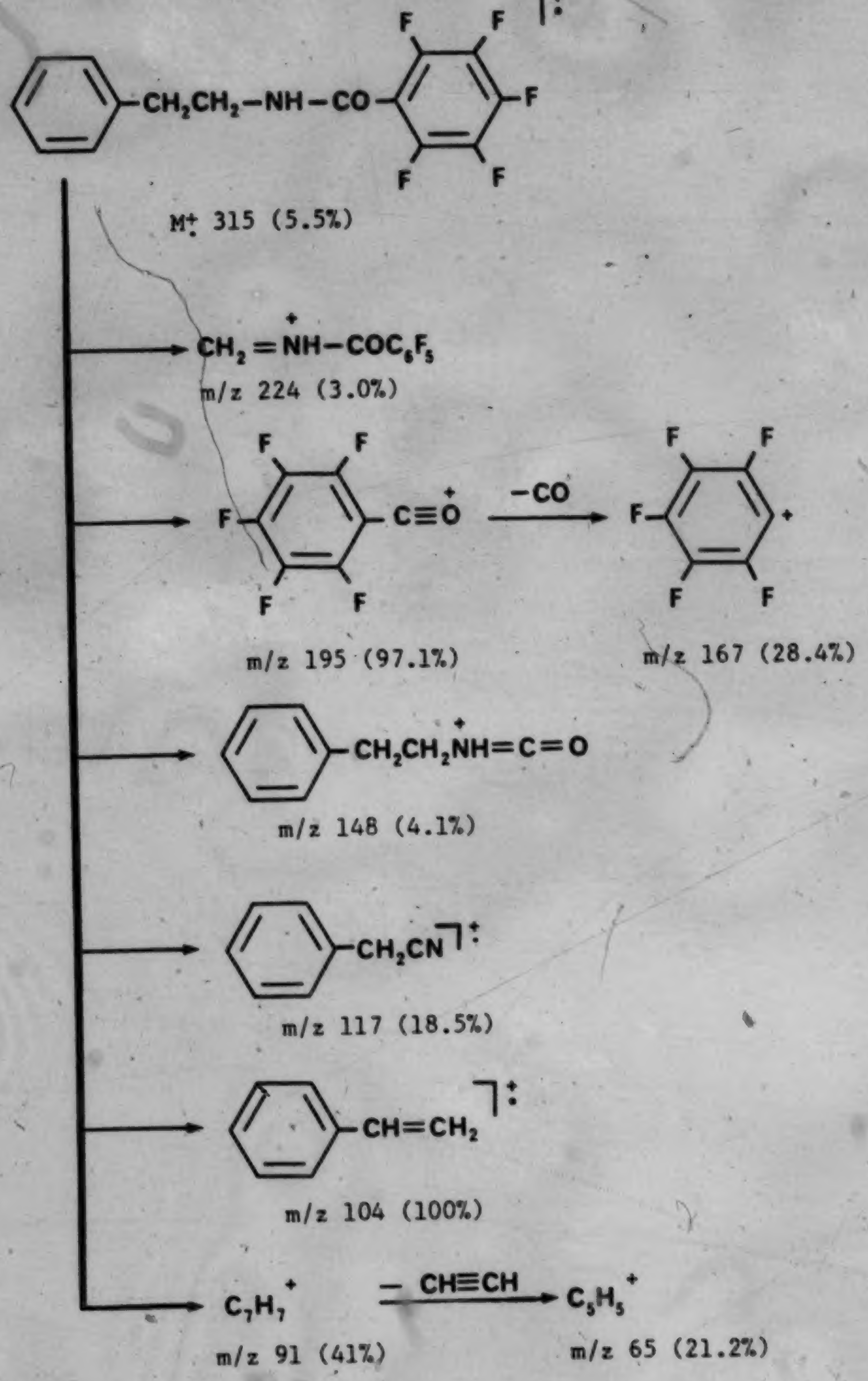

CH₂CH₂–NH–CO
F F F F F
M⁺ 315 (5.5%)
CH₂=NH–COC₆F₅
m/z 224 (3.0%)
C≡O
–CO
m/z 195 (97.1%)
m/z 167 (28.4%)
CH₂CH₂NH=C=O
m/z 148 (4.1%)
CH₂CN
m/z 117 (18.5%)
CH=CH₂
m/z 104 (100%)
C₇H₇⁺
– CH≡CH
C₅H₅⁺
m/z 91 (41%)
m/z 65 (21.2%)

Fig. 42. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of β-phenylethylamine.

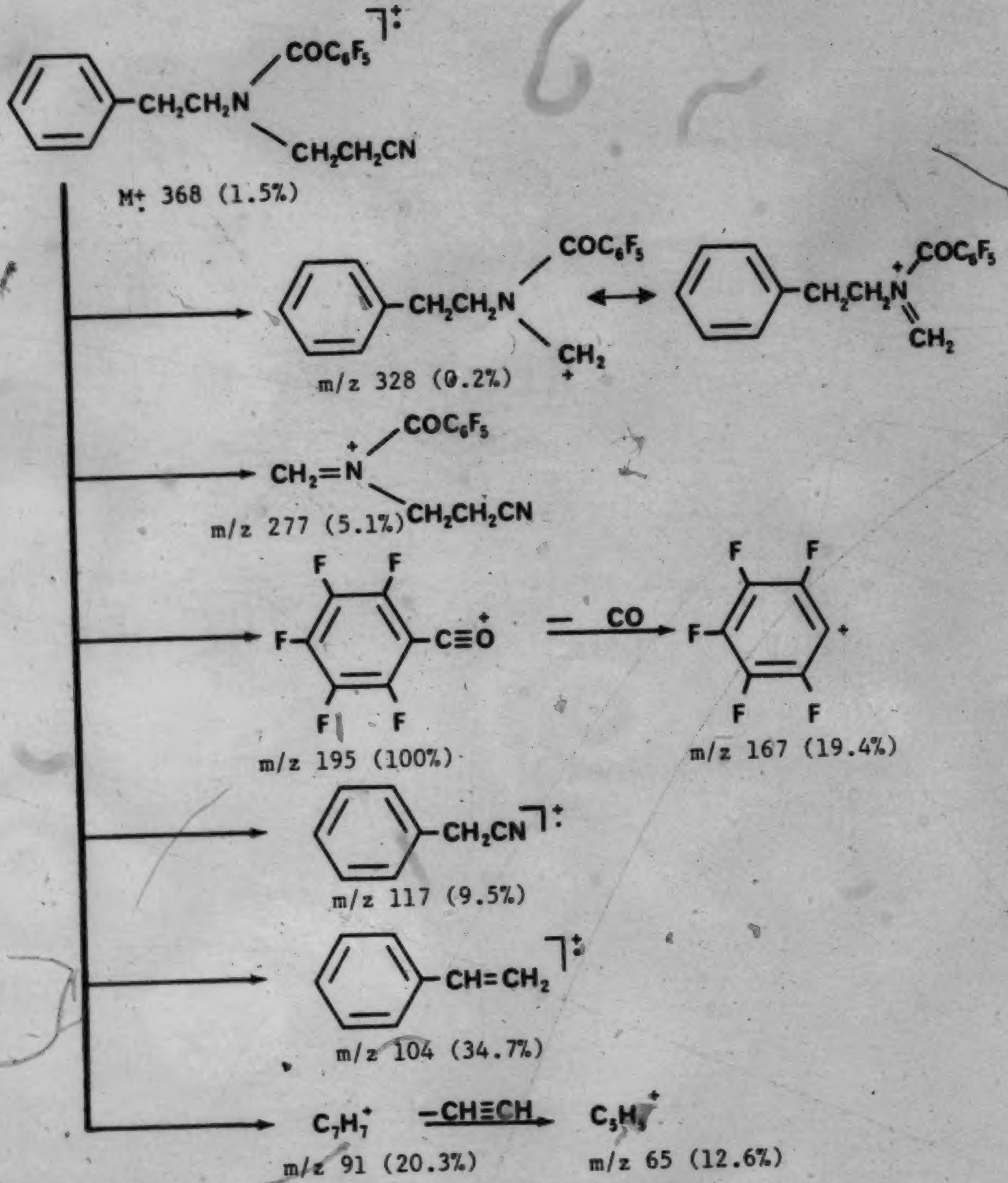

COC6F5
CH2CH2N
CH2CH2CN
M+ 368 (1.5%)
COC6F5
CH2CH2N
CH2
m/z 328 (0.2%)
COC6F5
CH2CH2N
CH2
COC6F5
CH2=N
CH2CH2CN
m/z 277 (5.1%)
F
C≡O
m/z 195 (100%)
− CO
m/z 167 (19.4%)
CH2CN
m/z 117 (9.5%)
CH=CH2
m/z 104 (34.7%)
C7H7
−CH≡CH
C5H5
m/z 91 (20.3%)
m/z 65 (12.6%)

Fig. 43. Probable mass spectral fragmentation of pentafluorobenzoyl derivative of N-(2-cyanoethyl)phenylethylamine.

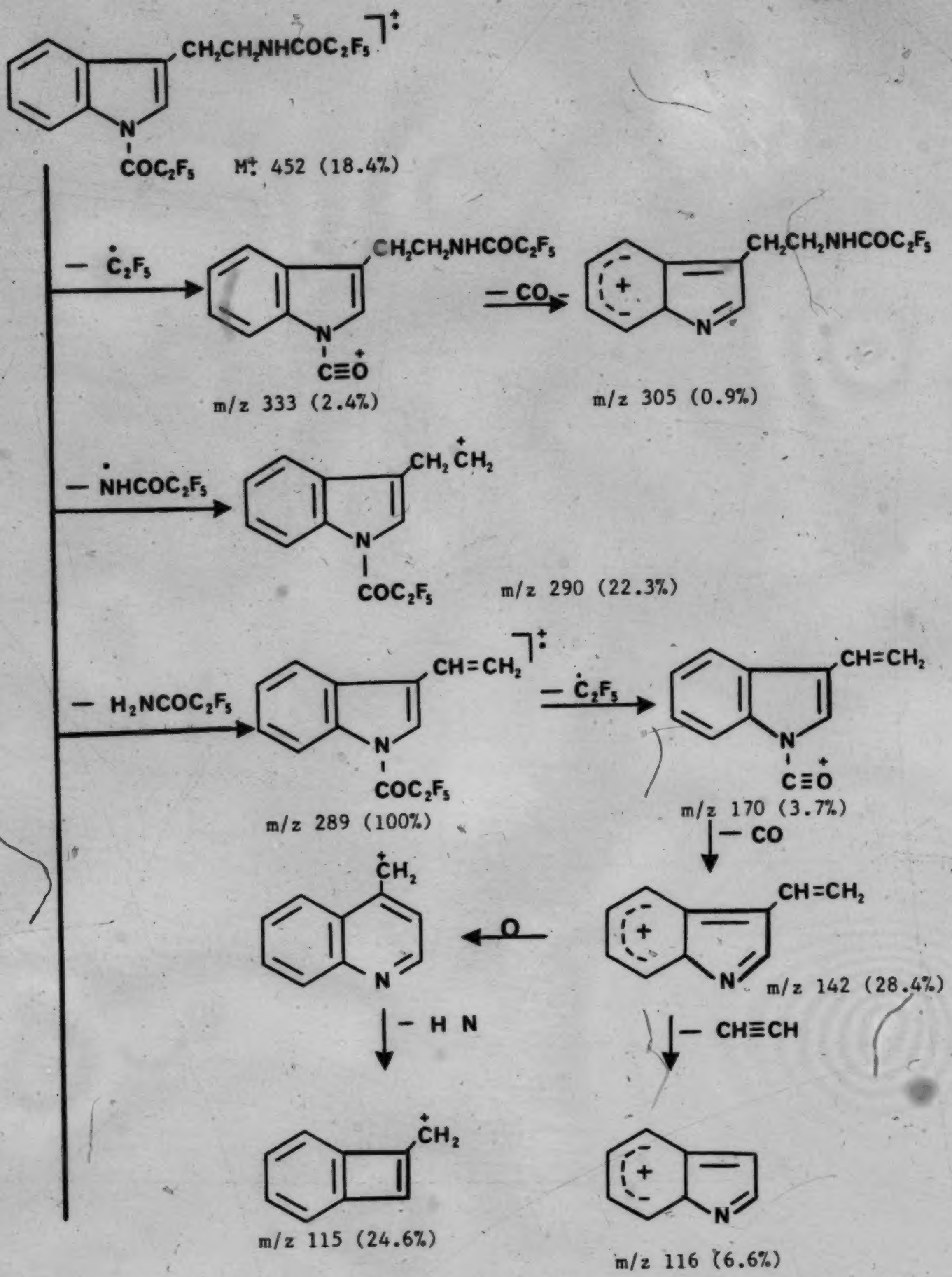

CH2CH2NHCOC2F5
N
COC2F5
M⁺˙ 452 (18.4%)
— Ċ2F5
CH2CH2NHCOC2F5
N
C≡O⁺
m/z 333 (2.4%)
— CO
CH2CH2NHCOC2F5
+
N
m/z 305 (0.9%)
— ṄHCOC2F5
CH2 ⁺CH2
N
COC2F5
m/z 290 (22.3%)
— H2NCOC2F5
CH=CH2
N
COC2F5
m/z 289 (100%)
— Ċ2F5
CH=CH2
N
C≡O⁺
m/z 170 (3.7%)
— CO
CH=CH2
+
N
m/z 142 (28.4%)
⁺CH2
N
— H N
⁺CH2
m/z 115 (24.6%)
— CH≡CH
+
N
m/z 116 (6.6%)

Fig. 44. Probable mass spectral fragmentation of pentafluoropropionyl derivative of tryptamine.

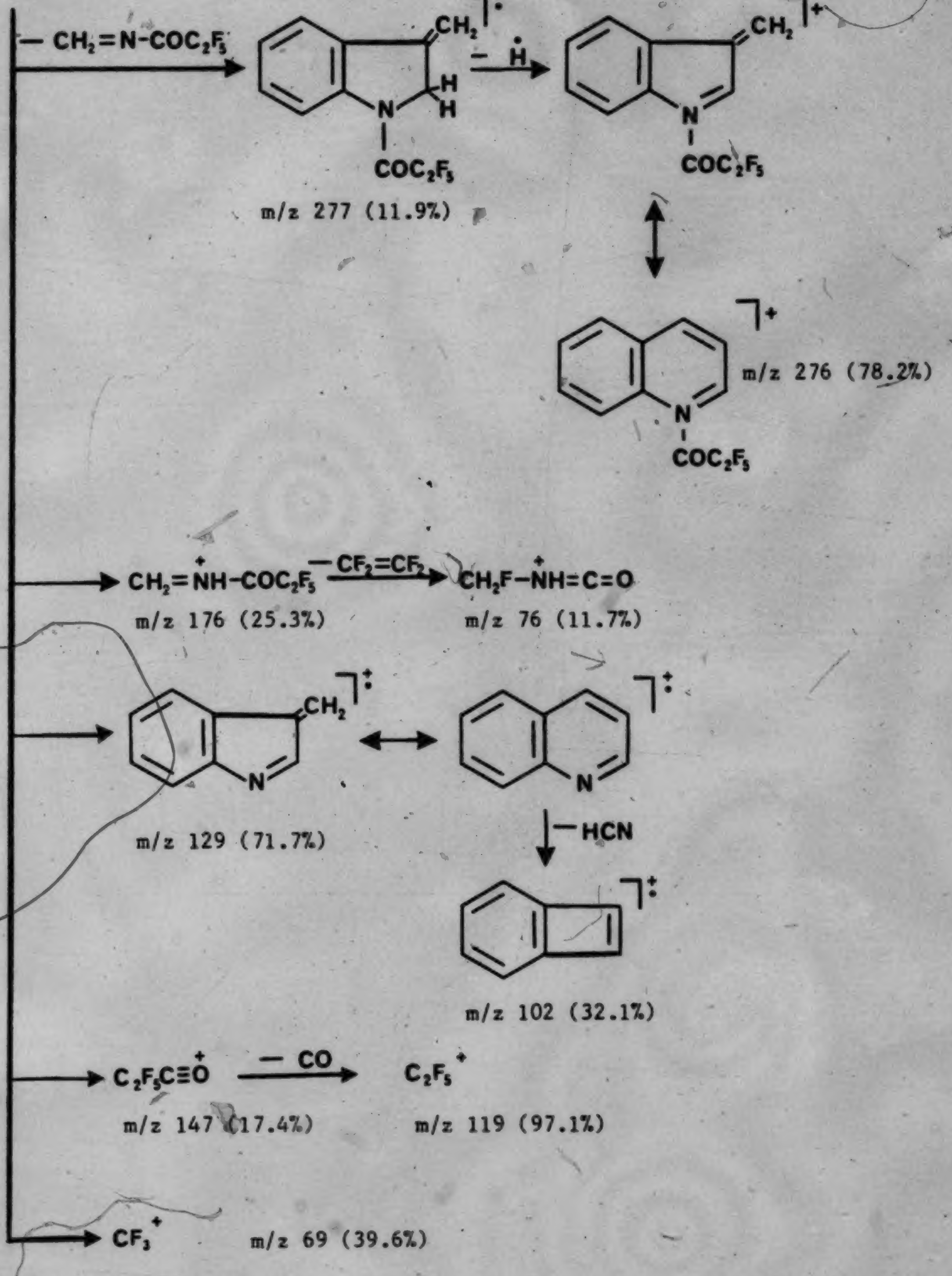

− CH₂=N-COC₂F₅
m/z 277 (11.9%)
− H•
m/z 276 (78.2%)
− CF₂=CF₂
CH₂=N⁺H-COC₂F₅
m/z 176 (25.3%)
CH₂F−N⁺H=C=O
m/z 76 (11.7%)
m/z 129 (71.7%)
− HCN
m/z 102 (32.1%)
− CO
C₂F₅C≡O⁺
m/z 147 (17.4%)
C₂F₅⁺
m/z 119 (97.1%)
CF₃⁺
m/z 69 (39.6%)

Fig. 44 (cont'd)

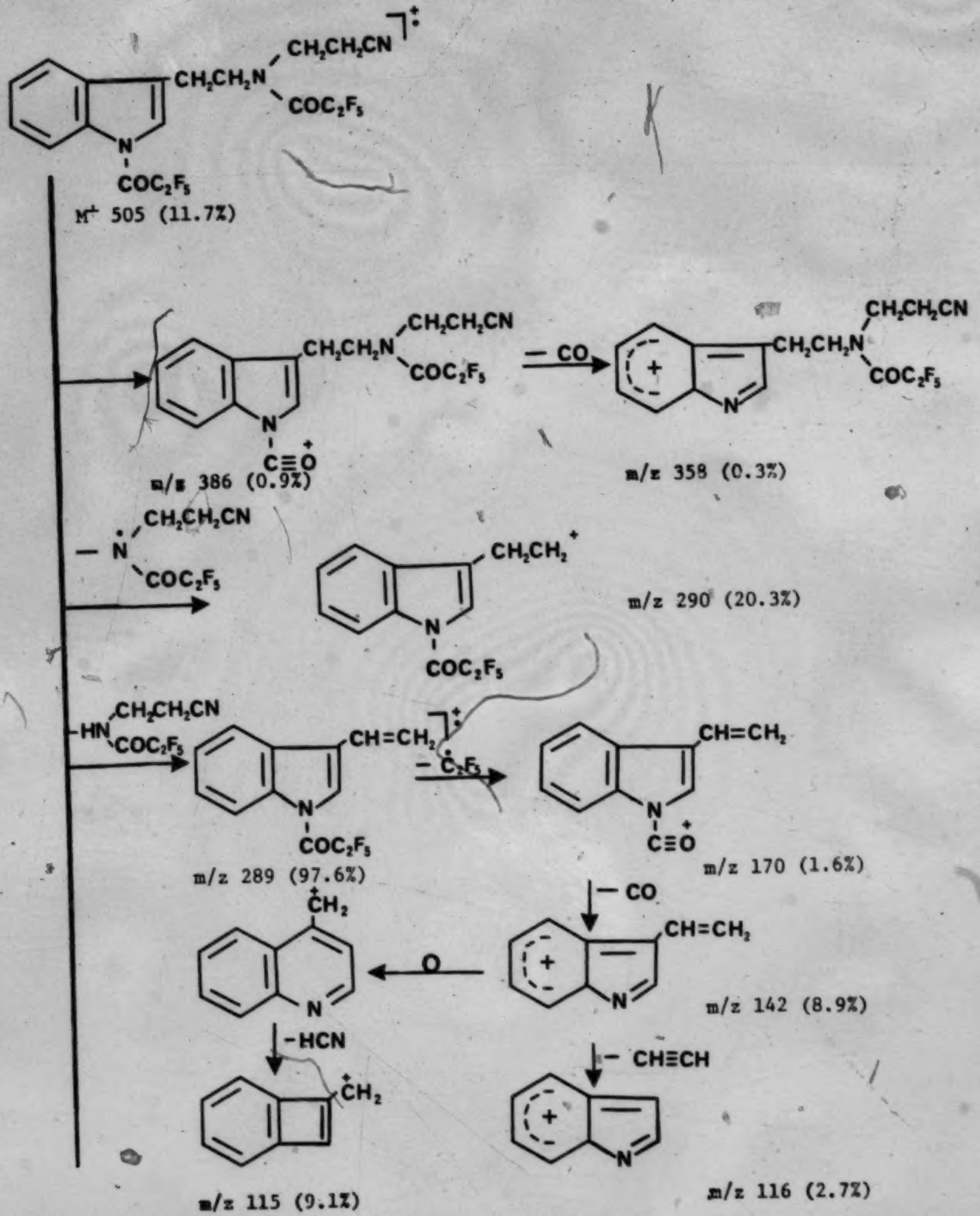

CH₂CH₂CN
CH₂CH₂N
COC₂F₅
N
COC₂F₅
M⁺ 505 (11.7%)
C≡O⁺
m/z 386 (0.9%)
− CO
m/z 358 (0.3%)
− N· CH₂CH₂CN COC₂F₅
CH₂CH₂⁺
m/z 290 (20.3%)
-HN CH₂CH₂CN COC₂F₅
CH=CH₂
m/z 289 (97.6%)
− ·C₂F₅
m/z 170 (1.6%)
− CO
m/z 142 (8.9%)
O
CH₂⁺
-HCN
m/z 115 (9.1%)
− CH≡CH
m/z 116 (2.7%)

Fig. 45. Probable mass spectral fragmentation of pentafluoropropionyl derivative of N-(2-cyanoethyl)tryptamine.

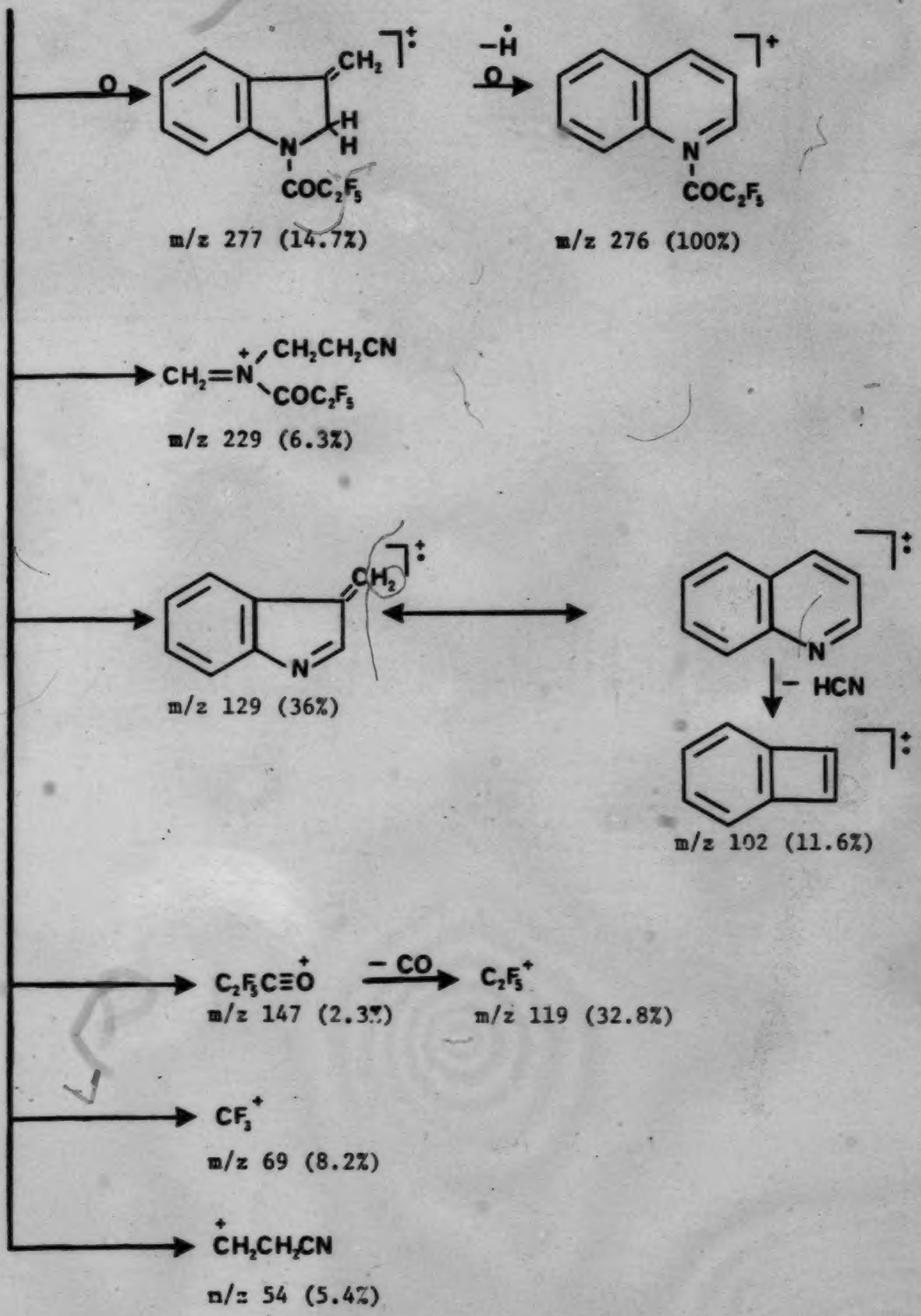

O
CH₂
H
H
N
COC₂F₅
m/z 277 (14.7%)
−Ḣ
O
N
COC₂F₅
m/z 276 (100%)
CH₂=N⁺ CH₂CH₂CN COC₂F₅
m/z 229 (6.3%)
CH₂
N
m/z 129 (36%)
N
− HCN
m/z 102 (11.6%)
C₂F₅C≡O⁺
m/z 147 (2.3%)
− CO
C₂F₅⁺
m/z 119 (32.8%)
CF₃⁺
m/z 69 (8.2%)
⁺CH₂CH₂CN
m/z 54 (5.4%)

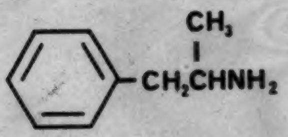
Fig. 45 (cont'd)

IV. DISCUSSION

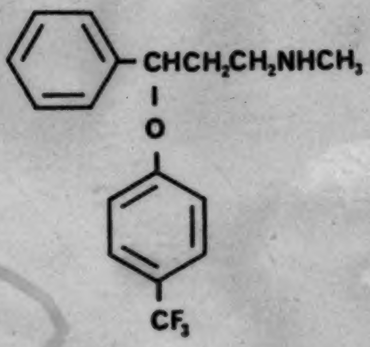
A. The Biochemistry and Pharmacology of Amphetamine and Analogues

Although AM has a simple chemical structure, it possesses a wide spectrum of pharmacological and biochemical activities. The structural features which appear to be critical for AM's activities are the α-methyl group, phenyl ring, primary amino group, and the two-carbon side chain between the phenyl ring and the nitrogen atom. The characteristic pharmacological action of AM is its central stimulatory activity. The α-methyl group as well as the β-phenylethylamine skeleton were found to be critical features for potent stimulant activity of the AM molecule (Van der Schoot _et al._, 1961). Other typical pharmacological properties of AM are anorexia (Cox and Maickel, 1972; Kuprys and Oltmans, 1982), vasoconstriction and hyperthermia (Biel and Bopp, 1978; Simpson, 1978). The biochemical action of AM involves the inhibition of NA and DA uptake (Burgen and Iversen, 1965; Coyle and Snyder, 1969; Taylor and Snyder, 1970; Steranka, 1983) as well as its ability to release these catecholamines from neurons (Daly _et al._, 1966; Taylor and Snyder, 1970; Masuoka _et al._, 1982). Amphetamine has a less marked effect on the serotoninergic neurons, although it has been reported to cause release of 5-HT from synaptosomes (Raiteri _et al._, 1977). _Para_-chloroamphetamine has strong effects on uptake and release of 5-HT, and can cause long-lasting depletion of brain 5-HT (Pletscher _et al._, 1964; Dewhurst and McKim, 1980).

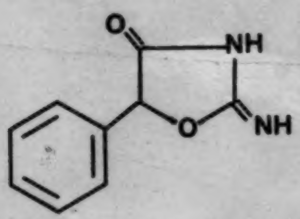
Amphetamine has been used clinically as an antidepressant and an anorexiant; however, chronic administration of AM can produce character-



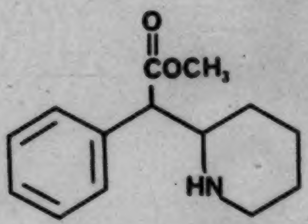
Amphetamine



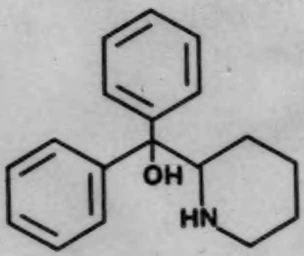
Fluoxetine (Lilly 110140)



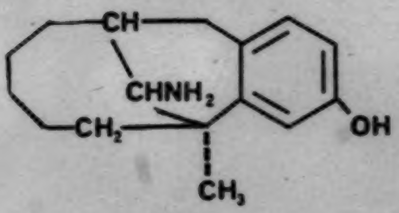
Pemoline



Methylphenidate



Pipradrol



Wy-16225

Fig. 46. Chemical structure of amphetamine and its synthetic derivatives.

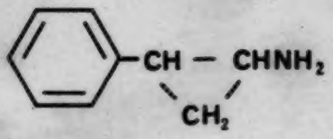
istic symptoms of paranoid psychosis (Ellinwood, 1967, 1968; Griffith et al., 1968). The stereotypical behaviour observed in animals after administration of AM, together with AM's ability to effect the release of DA from cerebral stores and inhibit its re-uptake, has given rise to the "dopamine hypothesis of schizophrenia." The facts that AM abuse can precipitate a model psychotic state indistinguishable from schizophrenia and that major antipsychotic drugs are known to be potent DA antagonists lend support to this hypothesis. Thus amphetamine exhibits many biochemical, pharmacological and physiological effects.

Due to the simplicity of AM's structure as well as its similarity to the biogenic amines of the CNS, neurochemists have employed AM as an effective tool to study the role of CNS biogenic amines in the aetiopathology of psychiatric disorders. Since AM displays multiple pharmacological and biological actions, it remains an important "model" drug for the medicinal chemist, providing a very versatile starting base for synthetic modifications. Structural changes in the AM molecule may accentuate or attenuate some of its effects, or even result in a novel property not found in the parent drug. Some examples of novel therapeutic agents which have resulted from the modification of the AM molecule are shown in Figure 46.

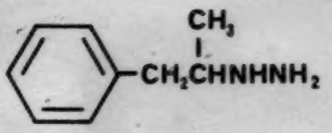
Fluoxetine (Lilly 110140) is a potential antidepressant, devoid of the anticholinergic properties of the tricyclics. It is a selective 5-HT uptake inhibitor (Fuller et al., 1974; Fuller and Wong, 1984). Pemoline has been used in the treatment of hyperkinetic syndrome or minimal brain dysfunction in children. It is a structurally modified AM with a carbonyl group in the α-position on the side chain. The side chain in turn is part of the oxazolidinone heterocyclic ring system

(Fig. 46). An important disadvantage of using AM and related stimulants is the development of tolerance and dependence (McCown and Barrett, 1980). Pemoline, however, does not have the capacity to reinforce self-administration behaviour in rhesus monkeys (Schuster et al., 1969; Dren et al., 1971, 1972), indicating that it does not produce dependence. The well known stimulants methylphenidate and pipradrol are cyclized AM derivatives containing a piperidine ring (Krueger and McGrath, 1964; Portoghese et al., 1968; Shafi'ee and Hite, 1969). A non-addicting analgesic agent, Wy-16225, is a cyclic analogue of AM (Fig. 46) (Biel and Bopp, 1978).

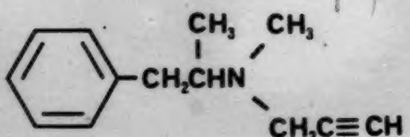
Relatively simple structural modifications in the AM molecule have resulted in three potent MAO inhibitors: TCP, pheniprazine, and deprenyl (Fig. 47). The α-methyl group is incorporated into the cyclopropane ring in TCP. This rigid structure exposes the amino group to the receptor and thereby facilitates binding. Tranylcypromine is about 5000 times more potent an MAO inhibitor than is AM (Burger and Yost, 1948; Zirkle and Kaiser, 1964; Fuller, 1972; Bieck and Antonin, 1982). Substituting the amino group with a hydrazine moiety in the AM molecule results in the potent MAO inhibitor pheniprazine. The hydrazine group is highly reactive, resulting in an irreversible inhibition of the MAO enzyme which can last for several days (Biel et al., 1964; Fuller, 1972). A similar substitution of the amino group with a hydrazine moiety in the β-phenylethylamine molecule leads to the formation of the MAO inhibitor phenelzine. Deprenyl is a selective MAO-B inhibitor in which a propargyl group is substituted on the amino group of the methamphetamine molecule. Deprenyl has an added advantage over other MAO inhibitors in that it does not potentiate tyramine-induced pressor effects.



Tranylcypromine



Pheniprazine

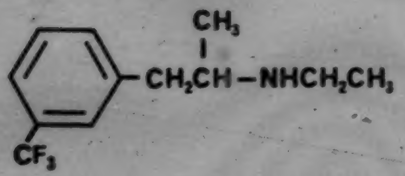


Deprenyl

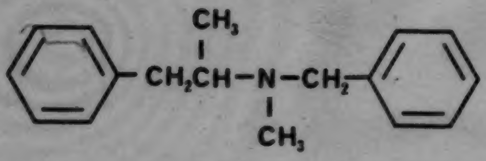
Fig. 47. MAO inhibitors with structural similarities to amphetamine.

Thus, the risk of a hypertensive crisis, which can occur with other MAO inhibitors as a result of concomitant ingestion of tyramine-rich foods such as cheese (Knoll and Magyar, 1972; Sandler et al., 1978), is avoided.

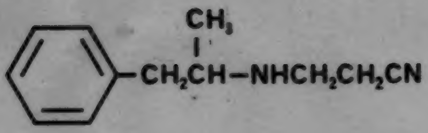
Although AM has prominent anorexiant properties, its use as an appetite suppressant is limited due to its central stimulatory effects. Separating its anorexiant property from stimulant effects would be desirable and much effort has been directed toward achieving this. A study conducted by Cox and Maickel (1972) with phenylethylamine derivatives which compared the anorexic and stimulant properties in rats gave some indication of the structural features required for selective anorexic property. The selectivity of the anorexic drug increased when there was (a) substitution of the phenyl ring with an electron-withdrawing group such as a chloro or a trifluoromethyl group (e.g. fenfluramine), (b) substitution on the amino group (e.g. benzphetamine, *p*-chlorobenzphetamine), and (c) dimethylation in the α-position (e.g. phentermine and chlorphentermine). Beregi et al. (1970) conducted a detailed structure-activity relationship study of trifluoromethylphenylisopropylamines. The presence of the α-methyl group and the separation of the amino group and the phenyl ring by no more than two carbons appeared to be essential for maximal anorexic property. Substitution on the amino group, forming mono-N-alkylated derivatives, resulted in the retention of a high anorexiant property with less of a vasopressor effect in rats. Substitution of the trifluoromethyl group in the *meta* position was most beneficial in retaining anorexic activity and also resulted in the loss of central stimulant effects. Chemical structures of some clinically used anorexic drugs which are derivatives of AM are


CH$_3$
CH$_2$CH–NHCH$_2$CH$_3$
CF$_3$

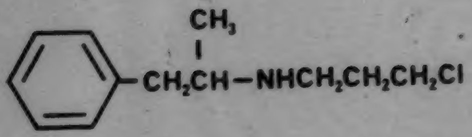
Fenfluramine (Ponderax®)


CH$_3$
CH$_2$CH–N–CH$_2$
CH$_3$

Benzphetamine (Didrex®)


CH$_3$
CH$_2$CH–NHCH$_2$CH$_2$CN

Fenproporex (Perphoxene®)


CH$_3$
CH$_2$CH–NHCH$_2$CH$_2$CH$_2$Cl

Mefenorex (Pondinil®)

Fig. 48. Chemical structures of some clinically used anorexic drugs which are derivatives of amphetamine.

illustrated in Figure 48. Recently Paul et al. (1982) have reported the presence of specific receptor sites in the rat hypothalamus which mediate the anorexic activity of AM and related drugs.

Thus it can be said that AM with its plethora of biochemical and pharmacological effects remains an important research tool. Moreover, it is a versatile starting base for the medicinal chemist in the design of novel pharmacological agents, providing an evergrowing armamentarium of therapeutic agents for the clinician.

B. Brain Concentrations of Bioactive Arylalkylamines and Analogues

B.1 Amphetamine and N-alkylated analogues

This study was conducted with the N-*n*-propyl, N-2-cyanoethyl, and N-3-chloropropyl analogues of AM. The objective of the study was to investigate the effect of the cyanoethyl, chloropropyl, or *n*-propyl side chain on the extent of *in vivo* metabolic dealkylation and to determine which group provided the highest concentration of AM in the brain. The reasons for employing these particular analogues will become clear further along in this discussion.

The N-(2-cyanoethyl)amphetamine (CE-AM, Perphoxene®) (Fig. 48), commonly known as fenproporex, is clinically used as an anorexiant in several European countries (Warembourg and Jaillard, 1968; Hertel and Fallot-Burghardt, 1978; Martindale, 1982a). *In vivo*, fenproporex has been found not to modify the catecholamine content of the myocardium or the adrenals of the rat (Cession-Fossion, 1970). Fenproporex has been recommended for treatment of obese patients with cardiovascular disease (Warembourg and Jaillard, 1968; Faivre et al., 1969) and is claimed to be devoid of the stimulant properties associated with AM (Vague et al., 1967; Plauchu et al., 1968). The implication of this claim is that an N-cyanoethyl substituent is resistant to metabolic removal, in contrast to an N-ethyl substituent which is readily dealkylated. Studies by others (Beckett et al., 1972; Tognoni et al., 1972) and the findings of the present study reported earlier (Nazarali et al., 1983) show, however, that the N-cyanoethyl substituent is readily removed metabolically.

The N-chloropropyl analogue of AM (CPA, Pondinil®) (Fig. 48), commonly known as mefenorex, is also a clinically used anorexiant (Beyer et al., 1980; Martindale, 1982a). The presence of the chloropropyl group is claimed to make this drug resistant to N-dealkylation since a substantial increase in para-hydroxylation of mefenorex relative to that seen with AM is observed in man and the rat, up to an extent of 37% and 58% respectively. It is a highly lipid-soluble amphetamine which is reported to be almost completely metabolized, with only 1% excreted unchanged in both man and the rat (Williams et al., 1973; Hirom and Smith, 1978; Caldwell, 1976). Mefenorex has been found to have no effect on pulmonary vascular resistance, either directly or indirectly, via a serotoninergic mechanism (Seiler et al., 1976). The metabolism of the N-n-propyl analogue of AM has been extensively investigated (Coutts et al., 1976a,b, 1978a,b, 1979, 1982; Coutts and Beckett, 1977; Coutts and Dawson, 1977; Coutts and Jones, 1982), and this amine was included in this study as a reference compound.

Metabolic studies of these drugs would reveal much information with respect to the bioavailability of AM to the brain. In view of the data obtained with these drugs, the feasibility of employing the cyanoethyl or the chloropropyl analogues of the biogenic amines PEA (which is similar in structure to AM) or T as possible pro-drugs of PEA and T could be investigated.

The biogenic amines PEA and T are present in trace amounts in the brain and have been implicated in several psychiatric disorders (Dewhurst, 1965, 1968; Fischer and Heller, 1972; Sandler and Reynolds, 1976; Boulton, 1980a; Sabelli et al., 1983). With the use of a pro-drug approach, it would be possible to provide a sustained release of these biogenic amines to the brain. It would also be possible to increase selectively the concentrations of these amines in the brain without affecting other endogenous amines. For instance, MAO inhibitors are used clinically as antidepressants but elevate the levels of all biogenic amines in brain, thereby confusing an understanding of their mode of action. By selectively elevating one specific amine with the use of a pro-drug approach, important information may be obtained with regard to the possible role of these amines in the aetiology of depression.

Before any investigation can be made on pro-drugs of bioactive amines, a suitable analytical procedure must be developed in order to quantitate the levels of the pro-drug and the parent amine in brain. Numerous derivatizing reagents and several analytical protocols were tested before adopting the present method of analysis. Aqueous pentafluorobenzoylation was found to give the best results for the simultaneous analysis of the pro-drug and the parent amine, in this case AM (Section II.E.1).

A number of methods have been used in the analysis of AM and analogues: spectrophotofluorometric (Nix and Hume, 1970; Hayes, 1973; Metha and Schulman, 1974), spectrophotometric (Stevens, 1973; Gill et al., 1982), thin-layer chromatographic (Kaistha and Jaffe, 1972; Bussey and

Backer, 1974; Decker and Thompson, 1978; O'Brien et al., 1982), gas-liquid chromatographic (Anggard et al., 1970; Schweitzer and Friedhoff, 1970; Driscoll et al., 1971; Clark, 1975; Jain, 1975; Souter, 1975; Beckett and Achari, 1977; Canfield et al., 1977; Clark, 1977; Terada et al., 1982; Martinez and Gimenez, 1983), high-pressure liquid chromatographic (Trinler et al., 1976; Clark et al., 1977; Kinberger, 1981; Farrell and Jefferies, 1983), mass spectrometric (Narasimhachari and Vourous, 1972; Cho et al., 1973; Cattabeni et al., 1974; Danielson and Boulton, 1974; Wu, 1975; Matin et al., 1977; Marde and Ryhage, 1978; Narasimhachari et al., 1979; Kojima et al., 1983), and radioimmunoassays (Mule et al., 1975; Digregorio and Kniaz, 1976; Powers and Ebert, 1979; Budd, 1981; Niwaguchi et al., 1982; Mason et al., 1983). In this study, a sensitive ECD-GLC procedure was employed. The technique developed for ECD-GLC use was rapid, sensitive, selective, and required a minimal clean-up procedure, and the primary (parent amine) and secondary (pro-drug) amines were simultaneously analysed after derivatization in aqueous medium (Section II.E.1). The concentrations of AM and its N-alkylated analogues were determined in rat brain by this method (Table IV). All three N-alkylated analogues--CE-AM (fenproporex), CPA (mefenorex), and NPA--were metabolised to AM in vivo. However, CE-AM gave the highest concentration of AM in brain at all three doses (0.05, 0.10 and 0.25 mmol/kg) compared to CPA or NPA. The analogues CPA and NPA gave about equal concentrations of AM in brain. The concentration of AM reached in brain after administration of CE-AM was significantly greater than that of CE-AM. This is in contrast to CPA or NPA where the concentrations of CPA or NPA in rat brain were much higher than their respective AM levels at all three doses. The results suggest that CE-AM has a very rapid rate of N-dealkylation in the rat, producing high concentrations of AM in brain. It is conceivable that the rat may be much more efficient than man in performing this dealkylation, but the significant amounts of AM found in human urine after oral administration of CE-AM (Beckett *et al.*, 1972) would seem to contradict this. Testa and Salvesen (1980) have observed that the N-dealkylation rate of N-substituted amphetamines increases with increasing chain length of the substrates; this may then explain the rapid N-dealkylation of CE-AM. The results also indicate that N-alkyl analogues may be good precursors of AM, bioactive amines, or related psychotropic drugs.

B.2 Time-concentration profile of amphetamine and its metabolite para-hydroxyamphetamine

In order to assess objectively the effect of the substituent (N-alkyl group), a time-concentration profile of AM in rat brain was conducted. Amphetamine as well as its major metabolite *p*-OH-AM were quantitated simultaneously in rat brain with ECD-GLC after aqueous pentafluorobenzoylation (Section II.E.1). Although AM and *p*-OH-AM have previously been measured in brain and various tissues (Axelrod, 1954; Belvedere *et al.*, 1973; Cho *et al.*, 1973; Danielson and Boulton, 1974; Jori and Caccia, 1974; Kreuz and Axelrod, 1974), Danielson and Boulton (1976) were the first to conduct a time-concentration profile of AM after intraperitoneal administration. However, half-lives and other pharmacokinetic data were not determined by these workers. The results of the study reported in this thesis (Fig. 15 and 16) indicate that the elimination half-life ($t_{1/2}$) of AM and *p*-OH-AM in rat brain were 43.0 and 100.3 min respectively. The area under the curve (AUC_{240}^{0}), which is an indication of bioavailability in brain, for AM and p-OH-AM were determined to be 17.55 and 0.43 μmol min/g respectively. The data also show that although p-OH-AM accumulation initially parallels AM levels in brain, there is a significant difference in its further accumulation and elimination. The concentration of AM in brain reaches a maximum at 30 min, whereas the concentration of p-OH-AM reaches a maximum at 60 min. This difference can be explained by the time lag required for the synthesis of p-OH-AM. However, a second explanation for this is that the responsible enzyme is subject to enzyme substrate inhibition, as is the case with hepatic enzymes (Jonsson, 1974; Cho et al., 1975). Hence p-OH-AM formation proceeds most readily when AM concentration is the lowest, which is initially during the absorption phase and finally during the elimination phase. Thus the concentration ratio of p-OH-AM to AM is the highest at these two phases of metabolism. The results correlate well with Danielson and Boulton's (1976) data. It seems paradoxical that the lipid soluble parent compound (AM) has a shorter half-life than the polar metabolite (p-OH-AM). This is due to the rapid accumulation of AM and its equally rapid efflux from tissues which follows distribution of a highly lipid soluble compound that does not have an active transport mechanism (Thoenen et al., 1968; Ross et al., 1968). Similar differences in half-lives of AM and p-OH-AM were observed by Taylor and Sulser (1973) after intraventricular administration. Moreover, electrical stimulation does not release AM from neurons, confirming that AM is not stored within neurons (Thoenen et al., 1966; Baldessarini, 1971). However, the metabolite p-OH-AM accumulates in catecholaminergic nerve terminals (Jori et al., 1979), which would explain its longer $t_{1/2}$. The enzyme dopamine-β-hydroxylase metabolizes p-OH-AM to para-hydroxynorephedrine (p-OH-NE), which competes with and replaces noradrenaline (NA) from neurons (Thoenen et al., 1966; Cavanaugh et al., 1970). This replacement of NA by a "false neurotransmitter" is claimed to be responsible for the development of tolerance to AM (Lewander, 1968; Brodie et al., 1970). However, tolerance to AM develops in guinea pigs, a species that does not produce any p-OH-NE (Sever et al., 1974, 1977). Amphetamine too can be β-hydroxylated to norephedrine, although AM is a poor substrate for the dopamine β-hydroxylase (Goldstein and Contrera, 1962). Although AM is metabolised to p-OH-AM in the liver (Jonsson, 1974; Cho et al., 1975), recent evidence indicates that synthesis can also occur in brain (Kuhn et al., 1978; Coutts et al., 1984b). The mechanism of p-OH-AM synthesis in brain is unknown; however, a recent report suggests that p-OH-AM is formed outside of the catecholamine neurons (Kuhn et al., 1978). Para-hydroxyamphetamine does not cross the BBB readily (Lindenbaum et al., 1975), and recent reports in which p-OH-AM levels were measured after i.p. injection of equimolar amounts of AM and p-OH-AM suggest that brain synthesis contributes significantly to the levels of p-OH-AM (Coutts et al., 1984b).

B.3 Time-concentration profile of N-(2-cyanoethyl)amphetamine and its metabolites amphetamine and para-hydroxyamphetamine

Of the three pro-drugs investigated, administration of N-(2-cyanoethyl)amphetamine (CE-AM, fenproporex) resulted in the highest amount of AM in rat brain after a single dose (Table IV). Hence a time-course evaluation of CE-AM and its metabolites AM and p-OH-AM in rat brain would give a good indication of the effect of the cyanoethyl group on the in vivo metabolism of CE-AM (Fig. 17 and 18). Analysis of all three amines, CE-AM, AM and *p*-OH-AM, was conducted simultaneously in rat brain with an ECD-GLC employing the novel procedure of aqueous pentafluorobenzoylation (Section II.E.1). Brain concentration of CE-AM reached maximum in the first 5 min, indicating a very rapid entry; however, an equally rapid efflux from brain occurred, with an exponential decline from 5 min to 120 min ($t_{1/2}$ = 21.5 min). This indicates that CE-AM, like AM, does not accumulate in brain and, like AM, it does not appear to have an active uptake mechanism (Thoenen *et al.*, 1968; Ross *et al.*, 1968). Amphetamine concentration reached maximum at 15 min, and AM levels remained higher than CE-AM at all times after 15 min. The bioavailability of AM (AUC_{240}^{0} = 8.66 µmol min/g) to rat brain after CE-AM was nearly half that obtained after an equimolar dose of AM (AUC_{240}^{0} = 17.55 µmol min/g). This indicates rapid N-dealkylation of CE-AM, that is, it is a good precursor of AM. The half-life ($t_{1/2}$) of AM in this study was determined to be 100.3 min, which is 2.3 times longer than the $t_{1/2}$ determined after administration of an equimolar dose of AM (for comparison see Fig. 15 and 17). These data support the hypothesis that the N-cyanoethyl analogue of AM is a useful pro-drug of AM.

An interesting observation was also made when the areas under the curve (AUC) of *p*-OH-AM after administration of CE-AM or AM alone were compared (Fig. 16 and 18). The area under the curve (AUC_{120}^{0}) of *p*-OH-AM in rat brain after administration of CE-AM was determined to be 0.35 µmol min/g and the AUC_{120}^{0} of *p*-OH-AM after an equimolar dose of AM was calculated to be 0.27 µmol min/g. This indicates that the bioavailability of *p*-OH-AM to the rat brain after CE-AM was greater from time period 0 to 120 min than that after administration of an equimolar dose of AM. Also the C_{max} (maximum concentration) of *p*-OH-AM after AM was 2.8 (±

0.9) nmol/g, whereas C_{max} of p-OH-AM after an equimolar dose of CE-AM was nearly 3.9 times higher at 10.8 (± 1.7) nmol/g (see Fig. 16 and 18). The t_{max}s (time at which concentration reaches a maximum) of the two time-concentration profiles were also different. After administration of CE-AM, the t_{max} of p-OH-AM in brain was 15 min, whereas the t_{max} of p-OH-AM after an equimolar dose of AM was as high as 60 min. A number of plausible factors can be put forward to explain these phenomena. Since the levels of AM in rat brain after administration of CE-AM were lower but remained sustained (as evidenced by longer $t_{1/2}$), it is unlikely that there was significant inhibition of the enzyme causing hydroxylation (Jonsson, 1974; Cho et al., 1975). Hence the enzyme responsible rapidly metabolized AM to p-OH-AM when a small but sustained concentration of AM was available.

Another possible explanation for the increased bioavailability of p-OH-AM to the brain after administration of CE-AM is that CE-AM may be metabolized to 4-hydroxy-N-(2-cyanoethyl)amphetamine (4-OHCE-AM) along with AM, and both 4-OHCE-AM and AM are in turn metabolised to p-OH-AM (Fig. 49). Hence two sources of p-OH-AM are available. A preliminary investigation with N-n-propylamphetamine (NPA) metabolism indicated the presence of p-hydroxy-N-n-propylamphetamine (p-OHNPA) in rat brain *in vivo*. Hence the possibility of p-hydroxylation of CE-AM cannot be ruled out, especially since the presence of p-OHNPA in human urine after administration of NPA has been shown by Coutts and Dawson (1977). Yet another explanation for increased bioavailability of p-OH-AM to the brain after CE-AM administration (in place of AM administration) is that CE-AM may in some way affect the metabolism of AM to p-OH-AM—that is, it may be an inducer of hydroxylating enzymes. All three possible

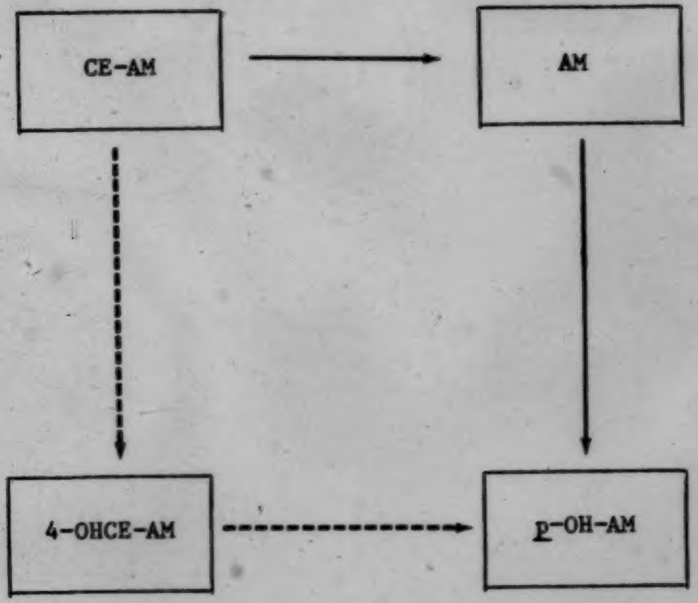


Fig. 49. Metabolic pathway of N-(2-cyanoethyl)amphetamine (CE-AM) to amphetamine (AM) and *p*-hydroxyamphetamine (*p*-OH-AM). The dotted line represents a "possible" alternate path leading to *p*-OH-AM via an intermediate metabolite, 4-hydroxy-N-(2-cyanoethyl)amphetamine (4-OHCE-AM).

explanations, the probable absence of substrate enzyme inhibition, the increased availability of *p*-OH-AM from two sources (4-OHCE-AM and AM), and the enzyme induction may contribute to the increased bioavailability of *p*-OH-AM after administration of CE-AM instead of AM.

More definitive answers to these questions will have to await further research on the enzyme(s) responsible for the hydroxylation. Detailed information on the amount of enzyme(s) present in the brain relative to other organs, on the subcellular distribution of the enzyme(s), and on the subcellular distribution of CE-AM and AM will be required to further understand the mechanisms involved.

The phenolic arylalkylamines such as *p*-OH-AM (and presumably also 4-OHCE-AM) do not penetrate the BBB readily (Lindenbaum *et al.*, 1975). Hence, the possibility that 4-OHCE-AM synthesis occurs in the brain must be considered, particularly in light of recent evidence in support of *p*-OH-AM synthesis in brain *in vitro* and *in vivo* (Kuhn *et al.*, 1978; Coutts *et al.*, 1984b). Since *p*-OH-AM is further hydroxylated to form 3,4-dihydroxyamphetamine (α-methyldopamine, "catecholamphetamine") (Daly *et al.*, 1965; Hoffman *et al.*, 1979), the possibility also exists that 3,4-dihydroxy-N-(2-cyanoethyl)amphetamine (3,4-diOHCE-AM) may be formed in brain. Coutts *et al.* (1976b, 1978a; Coutts and Dawson, 1977) first described the occurrence of 3-methoxy-4-hydroxy-N-*n*-propylamphetamine (3-MeO-4-OH-NPA) *in vivo* in rat and in man. Hence the possibility also exists that the catechol metabolite 3,4-diOHCE-AM, if present, is methylated by catechol-O-methyltransferase (COMT) to form 3-methoxy-4-hydroxy-N-(2-cyanoethyl)amphetamine (3-MeO-4-OHCE-AM). Since the catecholamine 3,4-dihydroxy-N-*n*-propylamphetamine was not detected in the urine of man or rat (Coutts *et al.*, 1976b; Coutts and Dawson, 1977), possibly due to its amphoteric nature and/or inefficient extraction, the novel method employing aqueous pentafluorobenzoylation or trichloroacetylation could be applied for selective extraction and identification of novel phenolic metabolites of N-alkylated amphetamines. The rapid disappearance of CE-AM from brain seen in the initial phase ($t_{1/2}$ = 21.5 min) may then be due to all these probable metabolic pathways of CE-AM discussed above as well as N-dealkylation (Fig. 17).

One final observation made in this study was that the $t_{1/2}$ of p-OH-AM after administration of AM was 100.3 min whereas the $t_{1/2}$ of p-OH-AM after an equimolar dose of CE-AM was 75.2 min (for comparison see Fig. 16 and 18). However, the C_{max} of p-OH-AM after CE-AM administration was 3.9 times higher than the C_{max} of p-OH-AM after an equimolar dose of AM. Yet the decline in p-OH-AM from the brains of CE-AM-treated animals was rapid (Fig. 18). An explanation for this phenomenon can be postulated. Since p-OH-AM accumulates in the catecholaminergic neurons (Jori et al., 1979), most p-OH-AM synthesized will probably find its way into these neurons. However, as the concentration of p-OH-AM increases, only so much of it can accumulate in the neurons. As the storage sites in the neurons get saturated, the excess p-OH-AM may undergo other metabolic routes or be eliminated since p-OH-AM molecules are polar and hydrophilic. It is interesting that the pattern of rapid decline of p-OH-AM levels is similar to that seen with CE-AM. These could be explanations for the shorter $t_{1/2}$ of p-OH-AM observed after administration of CE-AM.

B.4 Time-concentration profile of tranylcypromine

Tranylcypromine, a monoamine oxidase inhibitor, is a clinically used antidepressant (Martindale, 1982b). Although many reports of its toxicity in man have been documented (Baselt et al., 1977a; Youdim et al., 1979; Generali et al., 1981), studies on TCP's metabolic fate and pharmacokinetics have been neglected (Belanger, 1979; Hampson, 1984). Consequently, in contrast to AM, only a handful of methods for the analysis for TCP is found in the literature.

Turner et al. (1966) developed a fluorimetric procedure for analysis of TCP in urine. This method was subsequently employed in the study of the influence of pH on urinary excretion of TCP in man. Acidification of urine by prior administration of ammonium chloride resulted in as much as 11% of the dose of TCP being excreted unchanged (Turner et al., 1967). An enzymatic procedure based on transfer of ^{14}C-methyl of S-adenosyl-L-methionine-^{14}C to TCP in presence of rabbit lung N-methyltransferase was reported for the assay of TCP isomers in rat brain (Fuentes et al., 1975). Gas-liquid chromatographic methods employing flame-ionization detection (Baselt et al., 1977a), nitrogen detection (Bailey and Barron, 1980), and ECD (Baselt et al., 1977b; Calverley et al., 1981a) have also been used for the measurement of TCP.

A new ECD-GLC method was developed for the analysis of TCP in brain tissue. This method was highly sensitive (see Table VII), rapid, selective and reproducible. The acetylated pentafluorobenzoylated derivatives were stable at -20° for at least two months. An important advantage of this procedure was the simultaneous analysis of TCP and the trace amine PEA in brain. The method could also be adapted for the analysis of AM (Hampson et al., 1984a,b). The time-course of TCP in rat brain obtained by this method is shown in Figure 20. The data indicate that TCP enters the brain rapidly, reaching its peak concentration at 30 min. It is interesting to observe that AM after an equimolar dose also reaches its peak concentration at 30 min (Fig. 15). The difference between AM with its α-methyl group and TCP with the α-methyl incorporated into the cyclopropane ring would appear not to affect entry into the brain. Also the bioavailability to the brain of TCP (AUC_{240}^{0} = 15.8 μmol min/g) is not significantly different from that of AM (AUC_{240}^{0} = 17.55 μmol min/g). However, when the half-lives ($t_{1/2}$s) of TCP and AM are compared ($t_{1/2}$ of TCP = 100.3 min; $t_{1/2}$ of AM = 43.0 min), then it would appear that the cyclopropane ring of TCP has some effect on the slower clearance of TCP from brain (Fig. 15 and 20).

Although measurement of TCP in brain regions (Calverley et al., 1981a), comparison of the apparent antidepressant activity of (+)- and (-)-TCP (Fuentes et al., 1976), and the effect of TCP on biogenic amines of the brain (Philips and Boulton, 1979; Baker et al., 1984d) have been reported, a time-concentration profile of the clinically used racemic (±)-TCP in brain has not been conducted. Baselt et al. (1977b), however, investigated the time-course of (±)-TCP in man. The serum $t_{1/2}$ obtained from log-linear plot of data at 1, 3 and 5 hours correlated well with the $t_{1/2}$ of (±)-TCP obtained in rat brain. The rat therefore appears to be a good animal model with which to investigate pharmacokinetics of TCP in brain.

B.5 Time-concentration profile of N-(2-cyanoethyl)tranylcypromine and its metabolite tranylcypromine

The cyanoethyl analogue of AM was observed to have pronounced effect on the clearance of AM from brain (see Fig. 15 and 17 and discussion of half-lives on pages 148-152). A study was therefore initiated to investigate the effect of the cyanoethyl group on the clearance of TCP from brain. Although CE-AM is a clinically used anorexiant (Warembourg and Jaillard, 1968; Martindale, 1982a), the N-cyanoethyl analogue of TCP has never been tested for its pharmacological properties prior to this study. A number of analogues of TCP have been tested for their MAO inhibitory activity (Zirkle et al., 1962; Finkelstein et al., 1965; Teotino et al., 1967; McGrath and Kuhn, 1968), but their time-concentration profiles were not investigated. The MAO-inhibitory activities of these TCP analogues are discussed in Section IV.C.

In order to conduct time-concentration profiles of the novel analogue CE-TCP (see Fig. 12 for chemical structure) and its metabolite TCP in rat brain, sensitive analytical methods had to be developed. Aqueous pentafluorobenzoylation produced derivatives of both CE-TCP and TCP with good GLC-ECD sensitivity, but unfortunately a co-chromatographing peak from the brain interfered with the analysis. Acetylation in aqueous medium followed by pentafluorobenzoylation worked well for the sensitive analysis of TCP; however, TCP in presence of its analogue CE-TCP could not be sufficiently separated to give an accurate analysis. A method had to be found which would overcome these difficulties. A novel procedure employing trichloroacetic anhydride (TCAA) under aqueous conditions was eventually discovered to be the best analytical protocol. In this method, TCP as well as PEA could be analysed simultaneously, without interfering peaks, in brains of rats injected with CE-TCP. This novel procedure has a number of advantages. Although several methods (Baselt et al., 1977b; Martin and Baker, 1977; Calverley et al., 1981a; Hampson et al., 1984a,b) have been reported in the GLC analysis of TCP or PEA, all halogenated acyl derivatives were prepared under anhydrous conditions and in most cases required heat to complete the reaction. The present procedure, however, was successful under mild aqueous conditions. Direct aqueous acylation with TCAA produces a lipophilic derivative that is readily extracted into ethyl acetate. The method does not require an extensive clean-up procedure or the use of ion-pairing reagent. It is a rapid and highly sensitive procedure (5 pg "on-column" for PEA or TCP) which works for a number of primary and phenolic amines. A clean chromatogram was obtained (Fig. 50). Aqueous pentafluorobenzoylation was used for the analysis of CE-TCP. The structures of the derivatives formed under aqueous conditions were confirmed by mass-spectral analysis (Figs. 33 to 35). The time-concentration profiles of CE-TCP and its metabolite TCP are illustrated in Figure 21. It can be observed that the levels of TCP in brain remain higher than CE-TCP at all times, which suggests that CE-TCP is a good source of TCP. It is interesting to compare the rapid entry of CE-AM with a t_{max} of 5 min, as well as its initial rapid efflux from brain (Fig. 17) to the steady entry of CE-TCP with a t_{max} of 60 min and its somewhat slower clearance from brain (Fig. 21). Thoenen _et_ _al._ (1968) and Ross _et_ _al._ (1968) have reported that highly lipid soluble compounds which show rapid accumulation followed by rapid efflux from tissues do not have an active transport mechanism. This, then, suggests that CE-AM, like AM, does not have an active uptake mechanism. However, in contrast, CE-TCP has a gradual accumulation and a much slower clearance from the brain, but experimental evidence is lacking which would support an active transport for CE-TCP. It is possible that CE-TCP has a higher lipophilicity and accumulates in fatty tissue. This would explain the longer t_{max} value (60 min) as well as its slower clearance from brain.

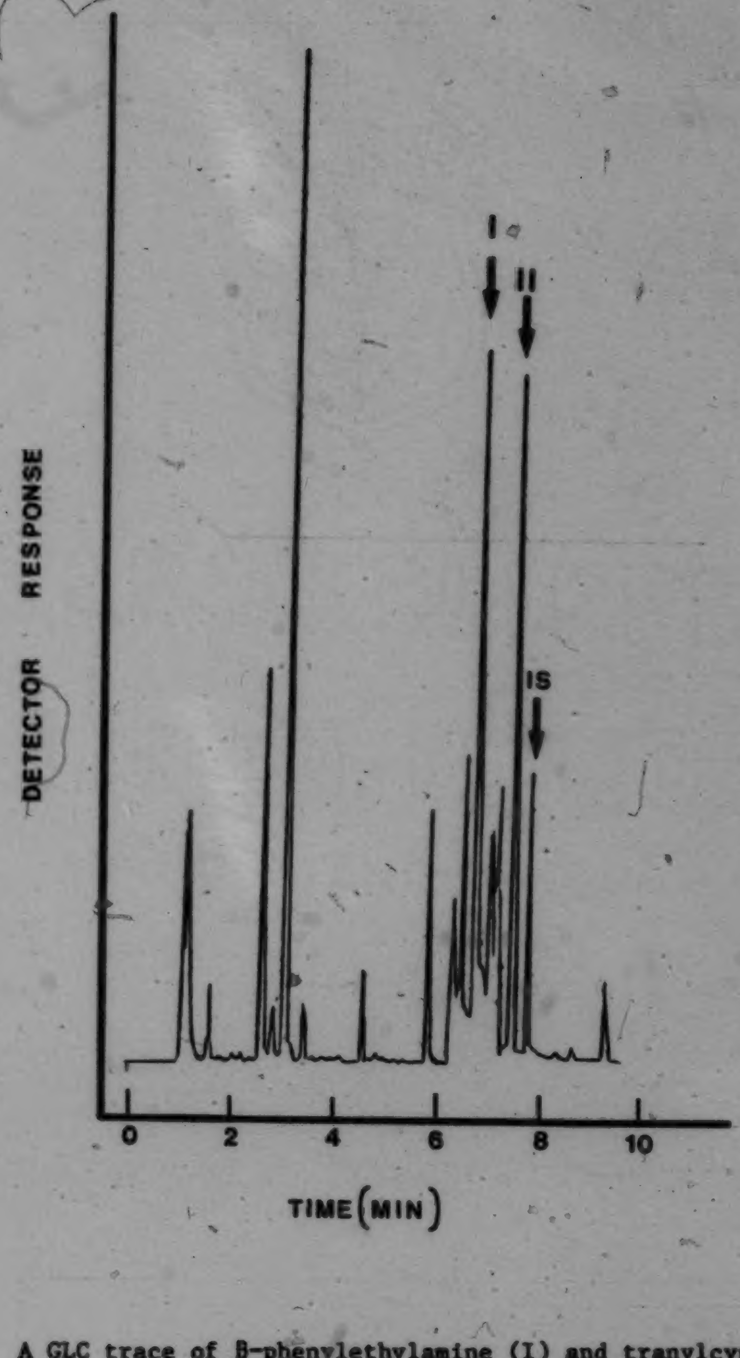


Fig. 50. A GLC trace of β-phenylethylamine (I) and tranylcypromine (II) after aqueous trichloroacetylation. Internal standard (IS) is p-chlorophenylethylamine.

The $t_{1/2}$ of TCP from brain after administration of CE-TCP was determined to be 150.5 min, whereas an equimolar dose of TCP had a $t_{1/2}$ of 100.3 min (Fig. 20 and 21). Therefore the cyanoethyl substituent affects the clearance of TCP from the brain and may be a good source of TCP. Recently, Hampson (1984) was able to detect the presence of *p*-hydroxytranylcypromine (*p*-OH-TCP) in brain and urine. Unfortunately, at the time this study was conducted, a synthetic sample of *p*-OH-TCP was not available. The novel methods employing aqueous pentafluorobenzoylation (successfully used in the analysis of *p*-OH-AM, Fig. 16 and 18) or aqueous trichloroacetylation could then have been applied for its analysis and a time-concentration profile conducted. The possibility of the metabolic production *in vivo* of 4-hydroxy-N-(2-cyanoethyl)tranylcypromine (4-OHCE-TCP) and 3-methoxy-4-hydroxy-N-(2-cyanoethyl)tranylcypromine (3-MeO-4-OH-CE-TCP) also exists, since Coutts *et al.* (1976b, 1978a; Coutts and Dawson, 1977) have described the presence of *p*-hydroxy-N-*n*-propylamphetamine (*p*-OHNPA) and 3-methoxy-4-hydroxy-N-*n*-propylamphetamine (3-MeO-4-OHNPA) *in vivo* in rat and in man after administration of NPA. The analytical methods developed could be adapted for the direct aqueous derivatization and efficient extraction of these possible phenolic metabolites.

Brain concentrations of CE-TCP and TCP after administration of [illegible]TCP were comparatively small. The areas under the curve (AUC_{240}^{0}) for CE-TCP and TCP were determined to be 1.15 μmol min/g and 1.55 μmol min/g respectively (Fig. 21). These values were significantly less than those obtained for CE-AM (AUC_{240}^{0} = 3.79 μmol min/g) and for AM (AUC_{240}^{0} = 8.66 μmol min/g) (Fig. 17). Therefore an equimolar dose of CE-AM compared to CE-TCP results in more parent amine in the brain. However, this does not mean that CE-TCP is a poor producer of TCP, since the AUC^{0}_{24} of TCP formed from CE-TCP is higher than that of CE-TCP. It can be postulated that CE-TCP may be bound to plasma proteins or accumulate in fatty tissue, and is therefore not easily accessible to metabolizing enzymes or able to pass through the BBB. This hypothesis would explain the smaller AUCs of CE-TCP and TCP, but further experimental evidence is required to substantiate this claim. An important observation from these studies is that the AUCs of TCP and AM are higher than those of their respective pro-drugs CE-TCP and CE-AM. These observations substantiate the initial hypothesis, which is that the N-cyanoethyl analogues may be useful pro-drugs of AM, TCP, and related psychotropic drugs or bioactive amines.

B.6 Preliminary investigation of the N-cyanoethyl analogues of β-phenylethylamine and tryptamine

Studies with the N-cyanoethyl analogues of AM and TCP have indicated that the cyanoethyl moiety is a useful substituent, which is metabolically removed to give AM and TCP in brain in pharmacologically active concentrations. These results were encouraging and prompted the investigation of the cyanoethyl analogue of PEA (which has structural similarities to AM and TCP) and T as possible pro-drugs of PEA and T. It has been reported that the concentrations of PEA and T in brain are elevated markedly by MAO inhibitors (Philips and Boulton, 1979). However, these drugs also elevate the brain concentrations of other amines, which confuses the treatment's mode of action. It is possible that PEA or T could be given by themselves, but since these amines are readily susceptible to metabolism by MAO, massive doses must be given to achieve reasonable brain concentrations. Under these conditions, the amines reach high levels in brain rapidly but also disappear extremely quickly (T. S. Rao, personal communication). Thus the pro-drug approach may represent a means by which less dramatically elevated, but more sustained, brain levels of these amines can be achieved. This may eventually enhance our understanding of the role(s) of PEA and T in affective disorders and may represent a novel means of treatment of such disorders.

Analytical methods were therefore required for the quantitation of CE-PEA and CE-T in brain (see Fig. 12 for chemical structure). Analysis of CE-PEA and its metabolite PEA was conducted with ECD-GLC in the same piece of brain tissue after aqueous pentafluorobenzoylation (Section II.E.1). Anhydrous pentafluoropropionylation was found to give the best results for the simultaneous analysis of the indoleamines CE-T and T (Section II.E.5). Numerous derivatizing reagents and analytical protocols were tested prior to adopting these methods. For example, aqueous pentafluorobenzoylation (aq. PFB); aqueous trichloroacetylation (aq. TCA); aq. TCA followed by anhydrous pentafluoropropionylation (anhyd. PFP); and aqueous acetylation (aq. AA) followed by anhyd. PFP were some of the derivatizing protocols tested for the analysis of the indoleamines. Derivatization employing aq. PFB did work, but broad peaks and long retention times were obtained. Tryptamine could be derivatized with aq. TCA, but the analogue CE-T was unreactive. Both aq. TCA and aq. AA followed by anhyd. PFP resulted in sensitive derivatives of T and CE-T, but unfortunately in both protocols the peaks of derivatized CE-T and T eluted too close to each other and prevented accurate analyses. Simultaneous analysis of CE-T and T was eventually obtained by employing

PFPA under anhydrous conditions to derivatize these amines.

Preliminary results shown in Table VI indicate that both CE-PEA and CE-T metabolize to PEA and T respectively, producing quantities of trace amines which were well above the control brain levels. Recent studies conducted by a colleague (Mr. T. S. Rao) with CE-PEA and CE-T administered to rats (without pretreatment with pargyline) also indicate that conversion of the analogues to their respective parent amines occurs. Mr. Rao's data indicates that CE-PEA rapidly enters the brain, reaching its C_{max} (maximum concentration) at 15 min after injection, but it also shows a rapid efflux from brain. It is interesting to observe that CE-AM in contrast reaches its C_{max} at 5 min, probably because of increased lipophilicity due to the α-methyl group.

These results indicate that administration of suitably N-substituted amines can alter the concentrations and time profiles of the parent amine in the brain. It is true that metabolism (N-dealkylation) of CE-PEA and CE-T may occur in the liver (first-pass metabolism), causing the biogenic amines to enter the systemic circulation and thence the brain. However, an important advantage of the pro-drug approach to increasing amine levels in the brain is that the MAO enzymes are not inhibited. Thus, excess amines in the circulation will be rapidly metabolized, reducing the risk of a hypertensive crisis, often associated with the use of MAO inhibitors. To avoid first-pass metabolism, a sublingual preparation of the pro-drug could always be considered for use. Data thus far show that CE-AM, CE-TCP, CE-PEA, and CE-T all enter the brain. There is a good chance that metabolism of these analogues also occurs in brain, since evidence shows that "the brain has conjugating systems present as well as those able to carry out Phase I metabolic pathways" (Gorrod, 1978).

B.7 Preliminary investigation of the N-ethoxycarbonyl analogues of amphetamine, β-phenylethylamine and tranylcypromine

An early study by Bjurulf et al. (1967) on the N-ethoxycarbonyl analogue of chlorphentermine (Oberex®) showed that a single dose of the pro-drug in the morning was sufficient to give a relatively prolonged drug effect. Several carbamate analogues (but not the N-ethoxycarbonyl analogue) of physiologically active amines, including AM, have been evaluated by Verbiscar and Abood (1970). In their study they report that "the anorexigenic action of the carbamates occurs with considerably less central stimulation than with amphetamine." The carbamates were also reported to provide a sustained effect. Hence the study on the effects of N-alkyl substituents on the ability of bioactive amines to enter and be cleared from the brain was extended to include the N-ethoxycarbonyl analogues of PEA, AM, and TCP (see Fig. 13 for chemical structures). The physiological basis for employing these analogues is discussed in Section D.2 of the Introduction.

Analysis was performed using ECD-GLC, with the amines and their N-ethoxycarbonyl analogues being converted to perfluoroacylated derivatives (Section II.E.4). Preliminary results (Table V) indicate that the N-ethoxycarbonyl analogues of AM and TCP are metabolized, as desired, to the parent amines AM and TCP. The concentrations of AM and TCP in brain were about twice as much as those of their respective analogues, which suggests a good conversion property. The N-ethoxycarbonyl analogue of PEA also gave a substantial quantity of PEA in brain which was about 12 to 19 times the normal control brain value. These results indicate that the N-ethoxycarbonyl analogues may prove to be useful pro-drugs of some bioactive amines, being able to alter the levels of these amines in, and their clearance from, brain, and therefore warrant further investigation.

C. Inhibition of Monoamine Oxidase

The MAO inhibitory activities of the cyanoethyl and ethoxycarbonyl analogues of the bioactive amines used in this study are illustrated in Table VIII. These analogues have not been previously tested for their activity against MAO. The cyanoethyl analogues of PEA, T and TCP, as well as the ethoxycarbonyl analogue of TCP, are novel drugs.

In vitro, the N-(ethoxycarbonyl)tranylcypromine (EthC-TCP) at a concentration of 4 μM inhibited MAO-A by 11.8 (± 2.7)% but had no effect on MAO-B. However, when EthC-TCP was administered i.p. to rats the inhibition of MAO-A and MAO-B in brain at 1 h was calculated to be 79.1 (± 3.4)% and 93.4 (± 0.8)% respectively (Table IX). Since it was determined that EthC-TCP gave high quantities of TCP (Table V), the equivalent concentrations of EthC-TCP and TCP found in brain *in vivo* were tested *in vitro*. The results (Table IX) show that EthC-TCP caused only 11.7 (± 4.1)% inhibition of MAO-A and no inhibition of MAO-B, which correlates well with the *in vitro* data in Table VIII. Tranylcypromine, on the other hand, caused significant inhibition of MAO-A and MAO-B under these conditions (Table IX). These results would indicate that EthC-TCP exhibits characteristics of a pro-drug, since the MAO inhibitory activity *in vivo* is due not to EthC-TCP but to the parent amine (TCP) formed from it.

The MAO inhibitory activity of several analogues of TCP have been tested. Zirkle et al. (1962) employed a pharmacological method to determine the MAO activity, relating it to the compound's ability to potentiate tryptamine-induced convulsions in rats. These workers have reported that the MAO inhibitory activity decreases when a substituent is introduced into the amino group, especially when large alkyl or arylalkyl groups are used. The N-methyl or N,N-dimethyl derivatives were found to be about half as active as TCP in the tryptamine potentiation test; however, the isopropyl derivative had only one hundredth the potency of TCP. Acylation of the amino group also caused a fall in potency, whereas the carbobenzoxy ($-CO_2CH_2C_6H_5$) analogue was reported to be twice as potent as TCP. A preliminary study conducted with the acetyl and the isopropyl derivatives of TCP in vivo (20 mg/kg, i.p. dose; rats killed 1.5 h after injection) indicated weak MAO-inhibitory activity. A modification of Wurtman and Axelrod's (1963) method (Section II.F) was used to measure the MAO activity. The percent MAO-inhibition of MAO-A and MAO-B in brain after N-acetyl-TCP administration was determined to be 29.0 (± 5.8) and 25.3 (± 8.5) respectively; after N-isopropyl-TCP administration, it was 24.6 (± 1.24) and 28.0 (± 4.9) respectively. These preliminary results indicate that the isopropyl and acetyl side chains are relatively resistant to N-dealkylation or deacetylation. However, the N-isobutylcarbamate analogue of TCP under the same conditions caused 58.8 (± 4.6)% and 77.6 (± 1.9)% inhibition of MAO-A and MAO-B respectively in vivo. This activity is probably due to the parent amine (TCP) being formed from the carbamate since little inhibitory activity was present in vitro.

Substitution on the phenyl ring was reported either to cause a decline in potency or to have little influence on tryptamine-potentiating activity (Zirkle _et al._, 1962). The _p_-trifluoromethyl and the _p_-methoxy derivatives of TCP were equipotent to TCP itself, indicating no difference between an electron-withdrawing or electron-donating group. Substitution in the order _para_ > _meta_ > _ortho_ position tended to increase the tryptamine-potentiating activity. A recent study (T. S. Rao, personal communication) indicates that _p_-fluorotranylcypromine is more potent than TCP against MAO-A and MAO-B _in vitro_.

The cyclopropane ring is an important feature for MAO activity (Zirkle _et al._, 1962; McGrath and Kuhn, 1968) since both _cis_- and _trans_-isomers of 2-phenylcyclobutyl-, 2-phenylcyclopentyl-, 2-phenylcyclohexyl- and 2-phenylcycloheptylamines were found to be inactive.

Of all the analogues tested in the present study, only CE-TCP had MAO-inhibiting activity _in vivo_ and _in vitro_ comparable to the clinically used TCP (Table VIII). The time-percent inhibition profile (Table X) of MAO in rat brain shows that both MAO-A and MAO-B are almost immediately inhibited after administration of CE-TCP. Both MAO-A and MAO-B remain inhibited by greater than 80% at 240 min. Inhibition of between 80 to 90% is widely accepted as the minimum required for therapeutic response (Robinson _et al._, 1978) as well as to affect amine levels (Green _et al._, 1977). Maximum inhibition occurs at 60 min, the time at which both CE-TCP and the parent amine (TCP) reach peak concentrations (Fig. 21). A parallel study with TCP (Table XI) also shows immediate inhibition of MAO-A and MAO-B with maximum inhibition occurring at 30 min, the time at which TCP concentration is the highest in brain (Fig. 20).

D. Brain Levels of β-Phenylethylamine, Tryptamine, and 5-Hydroxytryptamine after Administration of N-(2-Cyanoethyl)tranylcypromine

Data accumulated thus far show CE-TCP to be a potent MAO inhibitor both *in vitro* and *in vivo* (Tables VIII and X). It also forms substantial quantities of TCP in brain (Fig. 21). Further neurochemical studies were conducted to assess the status of monoaminergic systems in brain after administration of CE-TCP. A select group of biogenic amines, 5-HT (MAO-A specific substrate), PEA (MAO-B specific) and T (mixed substrate for MAO) were chosen for study. An active interest in these biogenic amines has been shown for some years primarily because of their possible role as neurotransmitters and neuromodulators and their possible involvement in the pathogenesis of psychiatric disorders. Numerous analytical methods have been developed for the quantitation of these biogenic amines for an assessment of their role in the CNS. Some of these methods are: spectrophotofluorometric (Cox and Perhach, 1973; Butterworth *et al.*, 1975; Suzuki and Yagi, 1976; Baker and Dewhurst, 1982), radioenzymatic (Saavedra, 1974; Hammel *et al.*, 1978; Martin, 1982; Saavedra, 1984), high-pressure liquid chromatographic (Sasa and Blank, 1977; Warsh *et al.*, 1979; Anderson and Young, 1981; Warsh *et al.*, 1982; Downer *et al.*, 1984), gas-liquid chromatographic (Edwards and Blau, 1972; Reynolds and Gray, 1976; Oliver *et al.*, 1977; Martin and Baker, 1977; Blau *et al.*, 1979; Calverley *et al.*, 1980; Hampson *et al.*, 1984a), and mass spectrometric (Durden *et al.*, 1973; Philips *et al.*, 1974; Warsh *et al.*, 1977; Artigas and Gelpi, 1979; Edwards *et al.*, 1979; Gelpi, 1982; Karoum, 1984).

In this study ECD-GLC has proven to be a sensitive and selective analytical tool for the quantitation of biogenic amines. The amines and their analogues were relatively easily derivatized with electron-capturing groups. Almost all conventional GLC methods involve preparation of derivatives under anhydrous conditions and in most cases in presence of heat. A novel procedure was developed in which a minimal clean-up procedure was required and the derivatives were prepared in mild aqueous conditions. Aqueous trichloroacetylation was employed in the simultaneous analysis of PEA and TCP (Section II.E.3). This method is highly sensitive and has many advantages over conventional GLC methods (see Section IV.B.5). A modification of the ECD-GLC procedure of Baker et al. (1980) (Section II.E.6) was employed for the analysis of 5-HT and T in the same piece of brain tissue. Results show that all three biogenic amines, PEA (Fig. 22), T (Fig. 23), and 5-HT (Fig. 24) are elevated in brain and remain elevated even at 240 min after administration of CE-TCP (Baker et al., 1984e). The brain levels of PEA, T and 5-HT at 240 min represent increases to approximately 100, 40 and 1.8 times the control values respectively. Interestingly, the increase in levels of the trace amines PEA and T were significantly greater than increase of 5-HT. Durden and Philips (1980) have reported that the dramatic elevation of trace amines seen after MAO inhibition may be due to their rapid turnover rates. In a novel theory of cerebral amine function, Dewhurst (1968) first described significant elevation of urinary T in depressed patients on an MAO inhibitor. Dewhurst (1968) suggested that T was a CNS transmitter in its own right after an extensive study in various animal species (rats, cats, guinea pigs, chickens and adult fowls) as well as in man (Dewhurst, 1961; Dewhurst and Marley, 1965). This view has been gaining support. The trace amines have been called neuromodulators since they have been shown to affect the post-synaptic actions of iontophoretically applied 5-HT (Jones and Boulton, 1980a) and DA (Jones and Boulton, 1980b). Murphy (1972) postulated that as the trace amines accumulate in the cytoplasm they enter storage vesicles where they displace conventional neurotransmitters or are released as co-transmitters. The ability of the trace amines to release conventional transmitters has been shown to occur (Baker and Yasensky, 1981b; Dyck, 1983). In an attempt to clarify the role of the trace amines, Boulton (1980b) suggested that they should be included as "neurohumors." The term means that the mechanism of action of trace amines may be involved either directly or indirectly in the process of neurotransmission. Dewhurst and Marley (1965) and later Jones (1981) demonstrated independent recognition sites (receptors) for T in the CNS. Intravenous administration of T to young chicks stimulated locomotor activity and electrocortical alerting, whereas 5-HT reduced locomotor activity (behavioural depression) and synchronized electrocortical activity. Recent in vitro binding studies (Hauger, 1982; Cascio and Kellar, 1983; R. A. Locock, personal communication) substantiate Dewhurst's early finding in that specific binding sites for both PEA and T have been reported in the CNS. The trace amines may well have a transmitter function. An imbalance in the neuronal homeostasis of the trace amines may have a profound effect on cerebral functions which might manifest behavioural dysfunctions seen in certain neuropsychiatric disorders (Dewhurst, 1968; Boulton and Milward, 1971; Sabelli and Mosnaim, 1974; Sandler and Reynolds, 1976; Boulton, 1980a). The present study has shown that the pro-drug CE-TCP is a potent MAO inhibitor in its own right which significantly elevates levels of PEA, T and 5-HT in brain in vivo. Since the cyanoethyl substituent has been used in a clinical situation in the past (e.g. the anorexiant fenproporex; CE-AM; see Section IV.B.1), the possibility exists for CE-TCP to be added to the armamentarium of therapeutic agents used in the treatment of affective disorders. However, further investigations are necessary before reaching such a conclusion. Importantly, this study indicates that effects on trace amines and on classical amines such as 5-HT should be conducted simultaneously in future investigations of such pro-drugs.

E. Brain Levels of β-Phenylethylamine, Tryptamine, and 5-Hydroxytryptamine after Administration of Tranylcypromine

An investigation of the effects of TCP on the *in vivo* brain concentrations of PEA, T, and 5-HT was conducted in a study parallel to that of the pro-drug CE-TCP. The brain levels of PEA (Fig. 25), T (Fig. 26) and 5-HT (Fig. 27) were approximately 45, 175 and 2.6 times the control values respectively at 240 min. However, the concentration of PEA had reached a maximum at 120 min (191.8 ± 20.1 ng/g) and its level had declined to 91.6 (± 16.4) ng/g, mean ± S.E.M., at 240 min. Similar findings have also been reported by Philips and Boulton (1979), but an explanation for the differential effect was not clarified. The reasons for the decline in PEA concentrations are not obvious. Maitre *et al.* (1976) have postulated the existence of two pools of MAO, the "bulk" mitochondrial MAO with a turnover of about 12 days (Gordis and Neff, 1971) and a smaller pool of MAO with a rapid turnover rate (12 to 18 hours). This smaller MAO pool has been claimed to be resistant to inhibition. A similar resistant amine oxidase has been reported to occur in the perfused rabbit lung together with two amine oxidases analogous to the A and B forms of the mitochondrial enzyme (Roth and Gillis, 1975). In light of these reports it is interesting to observe that the inhibition of MAO-A or MAO-B (Table XI) did not reach 100% at any time interval. The trend towards the decline of brain PEA would tend to support the hypothesis for the existence of a resistant pool of MAO. It is interesting that only PEA seems to be affected whereas 5-HT did not show a decline at 240 min, and leads one to postulate that perhaps this resistant pool of MAO may be primarily MAO-B. However, two important questions remain to be answered: (1) MAO-A inhibition does not reach 100% at any time interval and yet 5-HT levels remain elevated at 240 min; (2) T is a substrate for both MAO-A and MAO-B but its levels remain significantly above control at 240 min and do not show a decline.

Reports on brain MAO-A inhibition of 40-50% have been shown to be sufficient to substantially elevate brain 5-HT concentrations (Hampson, 1984) as well as to have a pharmacological effect (Luine and Paden, 1982). This would explain the elevated levels of 5-HT at 240 min since MAO-A was inhibited well over 80% at this time (Table XI). It would also partially explain the elevated levels of T, as T is a substrate for MAO-A too. It has been reported that administration of TCP causes an increase in brain concentrations of tryptophan (Grahame-Smith, 1971; Tabakoff _et_ _al._, 1977). Tryptophan is a precursor of both 5-HT and T, and may well significantly contribute to the elevated levels of 5-HT and T in the presence of inhibited MAO. The mechanism of this increase in brain tryptophan is unknown. Other MAO inhibitors have been known to influence amino acid transport (Rafaelson, 1976) and it may be that TCP facilitates tryptophan transport. It has also been reported that MAO inhibitors increase the aromatic amino acid decarboxylase activity in rat brain (Campbell _et al._, 1980). This fact, together with increased concentrations of tryptophan, would lead to increased brain levels of T.

An interesting observation was made in the case of the pro-drug CE-TCP. This potent MAO inhibitor caused no significant decline of PEA levels at 240 min. All three amines PEA, T and 5-HT remained elevated at 240 min. Although it is too early to make any conclusive inferences (a longer time study needs to be conducted), the present study reveals some interesting data. It is possible that the small pool of MAO (Maitre _et al._, 1976) is inhibited by CE-TCP (unlike TCP) and therefore prevents the decline of PEA. A second explanation may be that since CE-TCP forms substantial amounts of TCP in brain, the two MAO inhibitors could have a synergistic effect. Finally, the presence of novel metabolites of CE-TCP with MAO inhibitory activity cannot be excluded. Recently, Hampson (1984) identified the presence of _p_-OH-TCP in rat brain and urine. This metabolite was found to possess potent MAO inhibitory activity. Significant quantities of _p_-OH-AM were observed in rat brain after administration of CE-AM (Fig. 18). It is possible that high levels of _p_-OH-TCP could occur in brain after administration of CE-TCP. Coutts _et al._ (1976b, 1978a; Coutts and Dawson, 1977) identified 3-MeO-4-OH-NPA _in vivo_ in rat and in man following administration of NPA. This raises the possibility of the occurrence of a 3-methoxy-4-hydroxy derivative of CE-TCP which could have profound effects on the monoaminergic systems of the brain. Since the pro-drug CE-TCP did not have as dramatic an effect as TCP on the levels of T, it is possible that CE-TCP has little effect on tryptophan transport or on amino acid decarboxylase activity. However, it is possible that the increased brain bioavailability of TCP (AUC_{240}^{0} = 15.8 μmol min/g) (Fig. 20) compared to the combined brain bioavailability of CE-TCP (AUC_{240}^{0} = 1.15 μmol min/g) and TCP formed from it (AUC_{240}^{0} = 1.55 μmol min/g) (Fig. 21) may be responsible for higher levels of T.

It is clear that CE-TCP is a potent MAO inhibitor in its own right and has profound effects on the monoaminergic systems of the brain. Pro-drugs of this type seem well worthy of continuing investigations.

V. CONCLUSION

In the course of this study a number of significant advances were made in the development of gas-liquid chromatographic methods for the analysis of arylalkylamines present in a biological milieu. Analytical methods for fourteen arylalkylamines were developed employing the sensitive technique of ECD-GLC. Novel procedures employing direct derivatization in aqueous medium were successfully applied in the isolation, separation, identification, and quantitation of the arylalkylamines in brain. In the past, phenolic arylalkylamines have been analysed by lengthy procedures employing selective hydrolysis followed by perfluoroacylation under anhydrous conditions to achieve the desired sensitivity and gas-liquid chromatographic performance. The novel procedure of derivatizing directly in an aqueous environment with PFBC resulted in rapid and sensitive analysis of the phenolic arylalkylamine. The method has been employed for the simultaneous analysis of primary, secondary and phenolic arylalkylamines in the brain. Aqueous trichloroacetylation was employed in the analysis of PEA and TCP in the same piece of brain tissue. Structures of all final derivatives were confirmed by GLC-MS.

The methods of analysis developed in this study were rapid, selective and highly sensitive. They can be adapted for the sensitive analysis of a wide range of bioactive arylalkylamines and their analogues. The methods represent a major contribution to current neurochemical and drug metabolism investigations.

These analytical methods were successfully applied to the investigation of N-alkylated analogues which it was thought might be potential precursors of bioactive and biogenic amines. Substituents which were studied included the N-2-cyanoethyl, N-3-chloropropyl, N-n-propyl, and a variety of alkylcarbamates. However, the N-cyanoethyl derivatives proved most interesting and were studied in further detail.

N-(2-Cyanoethyl)amphetamine (Perphoxene®), CPA (Pondinil®), and NPA were shown to be metabolised to AM in vivo. Substantial concentrations of each analogue and AM were detected in brain. Of these three amphetamine pro-drugs, the analogue CE-AM gave the highest concentration of AM in brain at all three doses (0.05, 0.1, and 0.25 mmol/kg) studied. A time concentration profile of CE-AM in rat brain shows it to be a good source of AM. The half-life ($t_{1/2}$) and the C_{max} (maximum/peak concentration) of AM after administration of CE-AM was calculated to be 2.3 and 0.56 times the respective $t_{1/2}$ and C_{max} obtained after an equimolar dose of AM. The t_{max} (time at which concentration reaches a maximum) of AM after administration of CE-AM was half the t_{max} seen after an equimolar dose of AM. Results also indicate that CE-AM has a profound effect on the metabolic formation of p-OH-AM. The C_{max} of p-OH-AM in rat brain after administration of CE-AM was nearly 4 times higher and the t_{max} 4 times lower than the respective C_{max} and t_{max} of p-OH-AM calculated after an equimolar dose of AM.

N-(2-Cyanoethyl)tranylcypromine was found to be metabolized to TCP and high concentrations of CE-TCP and TCP were detected in the brain. The t_{max} of TCP in brain after administration of CE-TCP was double the t_{max} calculated after an equimolar dose of TCP. However, the C_{max} of TCP in brain was 8.35 times larger than the C_{max} of TCP obtained after an equimolar dose of CE-TCP. In addition, the $t_{1/2}$ of TCP in brain after administration of CE-TCP was about 1.5 times longer than the $t_{1/2}$ calculated for an equimolar dose of TCP. The concentrations of TCP in brain after i.p. administration of CE-TCP remain higher than CE-TCP at all times, which suggests that CE-TCP is a good precursor of TCP. Neurochemical studies show that CE-TCP is a potent MAO inhibitor in its own right in vitro and in vivo. It substantially elevates the levels of the biogenic amines 5-HT, T and PEA in brain in vivo. Interestingly, the increases in concentrations of the trace amines PEA and T were significantly greater than those observed for 5-HT. The results indicate that future investigations of such pro-drugs should involve studies on their effects on trace amines in addition to the more classical amines such as 5-HT.

The studies with the N-cyanoethyl analogues of AM and TCP have indicated that such derivatives can serve as effective precursors of AM and TCP in brain in pharmacologically active concentrations. These results prompted a preliminary investigation of the cyanoethyl analogue of the biogenic amines PEA and T as possible pro-drugs of PEA and T. The pro-drug approach is not novel in psychiatric research--long-acting neuroleptics have been successfully employed in stabilizing schizophrenic patients. However, this study is novel in taking a systematic approach in the analytical and neurochemical investigation of potential pro-drugs of bioactive and biogenic amines. The biogenic amines PEA and T are present in trace quantities in brain, and it has been suggested that a decreased functional availability of these amines may be a causative factor in depressive disorders. The concentrations of PEA have been reported to be elevated markedly by MAO inhibitors, but these antidepressant drugs also elevate the brain concentrations of other endogenous amines. A potentially important contribution to our understanding of the possible roles of amines in the biochemistry of affective disorders can be made by selectively elevating one specific amine such as PEA or T using a pro-drug approach. Investigations with the N-cyanoethyl and N-ethoxycarbonyl analogues of PEA and the N-cyanoethyl analogue of T have indicated substantial conversion of these analogues to parent amines.

The pro-drug approach is well worthy of continued effort and could provide a heuristic tool for the systematic investigation into the aetiopathology and treatment of affective disorders in psychiatry.

BIBLIOGRAPHY

Ahuja, S. (1976) Derivatization in gas chromatography. J. Pharm. Sci., 65: 163-182.

Albert, A. (1968) Absorption, distribution, and excretion, in Selective Toxicity, pp. 91-98. Butler and Tanner Ltd., London.

Albert, A. (1958) Chemical aspects of selective toxicity. Nature, 182: 421-423.

Anden, N. E., Corrodi, D. L., Dahlstrom, A., Fuxe, K., and Hokfelt, T (1966) Effects of tyrosine hydroxylase inhibition on the amine levels of central monoamine neurons. Life Sciences, 5: 561-568.

Anderson, G. M. and Young, J. G. (1981) Mini review: applications of liquid chromatographic-fluorometric systems in neurochemistry. Life Sci., 28: 507-517.

Anggard, E., Gunne, L. M., and Nicklasson, F. (1970) Gas chromatographic determination of amphetamine in blood, tissue and urine. Scan. J. Clin. Lab. Invest., 26: 137-143.

Anggard, E. and Hankey, A. (1969) Derivatives of sympathomimetic amines for gas chromatography with electron capture detection and mass spectrometry. Acta Chem. Scand., 23: 3110-3119.

Anggard, E. and Sedvall, G. (1969) Gas chromatography of catecholamine metabolites using electron capture detection and mass spectrometry. Anal. Chem., 41: 1250-1256.

Anton, A. H. and Sayre, D. F. (1962) A study of the factors affecting the aluminium oxide trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exptl. Therap., 138: 360-375.

Argauer, R. J. (1968) Rapid procedure for the chloroacetylation of microgram quantities of phenols and detection by electron capture gas chromatography. Anal. Chem., 40: 122-124.

Arnold, E. L. and Ford, R. (1973) Determination of catechol-containing compounds in tissue samples by gas-liquid chromatography. Anal. Chem., 45: 85-89.

Artigas, F. and Gelpi, E. (1979) A new mass fragmentographic method for the simultaneous analysis of tryptophan, tryptamine, indole-3-acetic acid, serotonin, and 5-hydroxyindole-3-acetic acid in the same sample of rat brain. Anal. Biochem., 92: 233-242.

Axelrod, J. (1954) Studies on sympathomimetic amines. II. The biotransformation and physiological disposition of d-amphetamine, d-p-hydroxyamphetamine and d-methamphetamine. J. Pharmacol. Exptl. Therap., 110: 315-326.

Bailey, E. and Barron, E. J. (1980) Determination of tranylcypromine in human plasma and urine using high resolution gas-liquid chromatography with nitrogen sensitive detection. J. Chromatogr., 183: 25-31.

Baker, G. B., Calverley, D. G., Dewhurst, W. G., and Martin, I. L. (1979) A sensitive gas chromatographic technique for quantification of urinary tryptamine. Brit. J. Pharmacol., 67: 469P-470P.

Baker, G. B., Coutts, R. T., and LeGatt, D. F. (1982a) Gas chromatographic analysis of amines in biological samples, in Analysis of Biogenic Amines (Baker, G. B. and Coutts, R. T., eds), pp. 109-125. Elsevier, Amsterdam.

Baker, G. B., Coutts, R. T., and Martin, I. L. (1981) Analysis of amines in the central nervous system by gas chromatography with electron-capture detection. Prog. Neurobiol., 17: 1-24.

Baker, G. B., Coutts, R. T., and Martin, I. L. (1984b) Gas chromatography for analysis of trace amines in tissues and body fluids, in Neurobiology of the Trace Amines (Boulton, A. A., Baker, G. B., Dewhurst, W. G., and Sandler, M., eds), pp. 57-68. Humana Press, Clifton, N.J.

Baker, G. B., Coutts, R. T., Nazarali, A. J., Danielson, T. J., and Rubens, M. (1984c) Carbamate prodrugs of phenylethylamines: a neurochemical investigation. Proc. West. Pharmacol. Soc., 27: 523-525.

Baker, G. B., LeGatt, D. F., and Coutts, R. T. (1984d) A comparison of the effects of acute and chronic administration of phenelzine and tranylcypromine on brain concentrations of 2-phenylethylamine, p-tyramine and tryptamine in the rat, in Neurobiology of the Trace Amines (Boulton, A. A., Baker, G. B., Dewhurst, W. G., and Sandler, M., eds), pp. 277-282. Humana Press, Clifton, N.J.

Baker, G. B., LeGatt, D. F., and Coutts, R. T. (1982c) A gas chromatographic procedure for quantification of para-tyramine in rat brain. J. Neurosci. Methods, 5: 181-188.

Baker, G. B., Martin, I. L., Coutts, R. T., and Benderly, A. (1980) Determination of 5-hydroxytryptamine in rat brain regions by gas-liquid chromatography with electron-capture detection. J. Pharmacol. Methods, 3: 173-179.

Baker, G. B., Martin, I. L., Coutts, R. T., and Benderly, A. (1982b) Para-chlorophenylethylamine in brains of rats treated with a monoamine oxidase inhibitor and p-chlorophenylalanine. Prog. Neuro-Psychopharmacol. & Biol. Psychiat., 6: 343-346.

Baker, G. B., Nazarali, A. J., and Coutts, R. T. (1984a) Aqueous trichloroacetylation and electron-capture gas chromatography for analysis of 2-phenylethylamine and tranylcypromine in brain tissue. Proc. Inter. Union Pharmacology, 1222P.

Baker, G. B., Nazarali, A. J., Coutts, R. T., Micetich, R. G., and Hall, T. W. (1984e) Brain levels of 5-hydroxytryptamine, tryptamine and 2-phenylethylamine in the rat after administration of N-cyanoethyltranylcypromine. Prog. Neuro-Psychopharmacol. & Biol. Psychiat., in press.

Baker, G. B. and Yasensky, D. L. (1981b) Interactions of trace amines with dopamine in rat striatum. Prog. Neuro-Psychopharmacol., 5: 577-580.

Baker, J. M. and Dewhurst, W. G. (1982) Fluorescence techniques for detection and quantitation of amines, in *Analysis of Biogenic Amines* (Baker, G. B. and Coutts, R. T., eds), pp. 63-81. Elsevier, Amsterdam.

Baldessarini, R. J. (1971) Release of aromatic amines from brain tissue of the rat *in vitro*. J. Neurochem., 18: 2509-2518.

Barbeau, A. (1967) The "pink spot" a 3,4-dimethoxyphenylethylamine and dopamine. Relationship to Parkinson's disease and to schizophrenia. Rev. Can. Biol., 26: 55-79.

Barbeau, A. (1969) L-Dopa therapy in Parkinson's diease: a critical review of nine years' experience. Can. Med. Ass. J., 101: 59-68.

Baselt, R. C., Shaskan, E., and Gross, E. M. (1977a) Tranylcypromine concentrations and monoamine oxidase activity in tissues from a fatal poisoning. J. Anal. Toxicol., 1: 169-170.

Baselt, R. C., Stewart, C. B., and Shaskan, E. (1977b) Determination of serum and urine concentrations of tranylcypromine by electron-capture gas-liquid chromatography. J. Anal. Toxicol., 1: 215-217.

Beckett, A. H. and Achari, R. (1977) Direct gas-liquid chromatographic analysis of N-hydroxyamphetamine, a metabolite of amphetamine. J. Chromatogr., 135: 200-204.

Beckett, A. H., Shenoy, E. V. B., and Salmon, J. A. (1972) The influence of replacement of the N-ethyl group by the cyanoethyl group on the absorption, distribution and metabolism of (±)-ethylamphetamine in man. J. Pharm. Pharmac., 24: 194-202.

Belanger, P. M. (1979) Electron impact induced fragmentation of some 1-substituted *trans*-2-phenylcyclopropane compounds. Biomed. Mass Spec., 6: 98-100.

Belvedere, G., Caccia, S., Frigerio, A., and Jori, A. (1973) A specific gas chromatogrpahic method for the detection of *p*-hydroxyamphetamine and *p*-hydroxynorephedrine in brain tissue. J. Chromatogr., 84: 335-360.

Benington, F., Christian, S. T., and Morin, R. D. (1975) Identification and separation of indolealkylamines by gas-liquid chromatographic analysis of their heptafluorobutyryl derivatives. J. Chromatogr., 106: 435-439.

Beregi, L. G., Hugon, P., LeDouarec, J. C., Laubie, M., and Duhault, J. (1970) Structure-activity relationships in CF_3 substituted phenylethylamines, in *Amphetamines and Related Compounds* (Costa, E. and Garattini, S., eds), pp. 21-61. Raven Press, New York.

Bertani, L. M., Dziedzic, S. W., Clarke, D. D., and Gitlow, S. E. (1970) A gas-liquid chromatographic method for the separation and quantitation of normetanephrine and metanephrine in human urine. Clin. Chim. Acta, 30: 227-233.

Bertler, A., Falck, B., Owman, C., and Rosengrenn, E. (1966) The localization of monoaminergic blood-brain barrier mechanisms. Pharmacol. Rev., 18: 369-385.

Beyer, G., Huth, K., Muller, G. M., Niemoller, H., Raisp, I., and Vorberg, G. (1980) The treatment of obesity with appetite-curbing agent Mefenorex. Med. Welt., 31: 306-309.

Bieck, P. R. and Antonin, K. H. (1982) Monoamine oxidase inhibition by tranylcypromine: assessment in human volunteers. Eur. J. Clin. Pharmacol., 22: 301-308.

Biel, J. H. and Bopp, B. A. (1978) Amphetamines: structure-activity relationships, in *Handbook of Psychopharmacology*, vol. 11 (Iversen, L. L., Iversen, S. D., and Snyder, S. H., eds), pp. 1-39. Plenum Press, New York.

Biel, J. H., Horita, A., and Drukker, A. E. (1964) Monoamine oxidase inhibitors (hydrazines), in *Psychopharmacological Agents*, vol. 1 (Gordon, M., ed.), pp. 359-443. Academic Press, New York.

Bjorkman, S., Idvall, J., and Stenberg, P. (1983) Gas-liquid chromatographic determination of methohexital in plasma or whole blood with electron capture detection of pentafluorobenzoyl derivative. J. Chromatogr., 278: 424-428.

Bjurulf, P., Carlstrom, S., and Rorsman, G. (1967) Oberex, a new appetite-reducing agent. Acta Medica Scandinavica, 182: 273-280.

Blau, K., Claxton, I. M., Ismahan, G., and Sandler, M. (1979) Urinary phenylethylamine excretion: gas chromatographic assay with electron-capture detection of the pentafluorobenzoyl derivatives. J. Chromatogr. Biomed. Appl., 163: 135-142.

Blau, K. and King, G. S. (1978) Acylation, in *Handbook of Derivatives for Chromatography* (Blau, K. and King, G. S., eds), pp. 104-151. Heyden, London.

Bock, U. E. G. and Waser, P. G. (1981) Gas chromatographic determination of some biogenic amines as their pentafluorobenzoyl derivatives in the picogram range and its applicability to biological materials. J. Chromatogr., 213: 413-428.

Bruce, R. B. and Maynard, W. R. Jr. (1969) Determination of amphetamine and related amines in blood by gas-chromatography. Anal. Chem., 41: 977-979.

Budd, R. D. (1981) Amphetamine radioimmunoassay—structure versus reactivity. Clin. Toxicol., 18: 299-316.

Burgen, A. S. V. and Iversen, L. L. (1965) The inhibition of norepinephrine uptake by sympathomimetic amines in the rat isolated heart. Brit. J. Pharmacol., 25: 34-39.

Burger, A. and Yost, W. L. (1948) Arylcycloalkylamines. I. 2-Phenylcyclopropylamine. J. Am. Chem. Soc., 70: 2198-2201.

Bussey, R. J. and Backer, R. C. (1974) Thin-layer chromatographic differentiation of amphetamine from other primary-amine drugs in urine. Clin. Chem., 20: 302-304.

Butterworth, R. F. (1983) Amino acid neurotransmitter function in thiamine deficiency encephalopathy. J. Neurochem., 41: Suppl. S31D.

Butterworth, R. F., Landreville, F., Guitard, M., and Barbeau, A. (1975) A reliable method for the simultaneous estimation of dopamine, noradrenaline and serotonin in discrete areas of brain. Clin. Biochem., 8: 298-302.

Caldwell, J. (1976) The metabolism of amphetamines in mammals. Drug Metab. Rev., 5: 219-280.

Calne, D. B., Karoum, F., Ruthven, C. R. J., and Sandler, M. (1969) The metabolism of orally administered L-dopa in Parkinsonism. Brit. J. Pharmacol., 37: 57-68.

Calverley, D. G., Baker, G. B., Coutts, R. T., and Dewhurst, W. G. (1981a) A method for measurement of tranylcypromine in rat brain regions using gas chromatography with electron-capture detection. Biochem. Pharmacol., 30: 861-867.

Calverley, D. G., Baker, G. B., McKim, H. R., and Dewhurst, W. G. (1980) A gas chromatograph technique using electron-capture detection for simultaneous estimation of tryptamine and 5-hydroxytryptamine in biological tissue. Can. J. Neurol. Sci., 7: 237.

Calverley, D. G., McKim, H. R., and Dewhurst, W. G. (1981b) Inhibitory effect of some monoamine oxidase inhibitors on fluorescence assay for the O-methylated metabolites of the catecholamines. J. Pharmacol. Methods, 5: 179-181.

Campbell, I. C., Murphy, D. L., Walker, M. N., Lovenberg, W., and Robinson, D. S. (1980) Monoamine oxidase inhibitors (MAOI) increase rat brain aromatic amino acid decarboxylase activity. Brit. J. Clin. Pharmacol., 9: 431-432.

Boireau, A., Ternaux, J. P., Bourgoin, S., Hery, F., Glowinski, J., and Hamon, M. (1976) The determination of picogram levels of 5-HT in biological fluids. J. Neurochem., 26: 201-204.

Bond, P. A. (1972) The determination of 4-hydroxy-3-methoxyphenylethylene glycol in urine and CSF using gas chromatography. Biochem. Med., 6: 36-45.

Borgman, R. J., McPhillips, J. J., Stitzel, R. E., and Goodman, I. J. (1973) Synthesis and pharmacology of centrally acting dopamine derivatives and analogs in relation to Parkinson's disease. J. Med. Chem., 16: 630-633.

Boulton, A. A. (1980b) The properties and potential function of some brain trace amines, in Neurochemistry and Clinical Neurology (Alan, R., ed.), pp. 291-303. Liss Inc., New York.

Boulton, A. A. (1980a) Trace amines and mental disorders. Can. J. Neurol. Sci., 7: 261-263.

Boulton, A. A. and Milward, L. (1971) Separation, detection and quantitative analysis of urinary 2-phenylethylamine. J. Chromatogr., 57: 287-296.

Boulton, A. A., Philips, S. R., Durden, D. A., Davis, B. A., and Baker, G. B. (1976) The tissue and cerebral subcellular distribution of some arylalkylamines in the rat and the effect of certain drug treatments on these distributions. Adv. Mass Spec. Biochem. Med., 1: 193-205.

Braestrup, C. (1973) 3-Methoxy-4-hydroxyphenylethanol in the rat brain. J. Neurochem., 20: 519-527.

Brenner, N. and Olson, R. J. (1967) Basic knowledge of gas chromatography and gas chromatographic instruments, in The Practice of Gas Chromatography (Ettre, L. S. and Zlatkis, A., eds), pp. 1-49. Interscience, New York.

Brodie, B. B., Cho, A. K., and Gessa, G. L. (1970) Possible role of p-hydroxynorephedrine in the depletion of norepinephrine induced by d-amphetamine and in tolerance to this drug, in International Symposium on Amphetamines and Related Compounds (Costa, E. and Garattini, S., eds), p. 217. Raven Press, New York.

Brodie, B. B., Kurz, H., and Schanker, L. S. (1960) The importance of dissociation constant and lipid-solubility in influencing the passage of drugs into the cerebrospinal fluid. J. Pharmacol. Exptl. Therap., 130: 20-25.

Brooks, C. J. W. and Horning, E. C. (1964) Gas chromatographic studies of catecholamines, tryptamines, and other biological amines. Part I. Catecholamines and related compounds. Anal. Chem., 36: 1540-1545.

Canfield, D. V., Lorimer, P., and Epstein, R. L. (1977) Gas chromatographic analysis of amphetamine derivatives and morpholine-related drugs. J. Forensic Sci., 22: 429-433.

Cascio, C. S. and Kellar, K. J. (1983) Characterization of ^{3}H-tryptamine binding sites in brain. Eur. J. Pharmacol., 95: 31-39.

Cattabeni, F., Racagni, G., and Paoletti, R. (1974) A mass-fragmentographic study of amphetamine and metabolites in rat brain. J. Psychiatr. Res., 11: 45-51.

Cavanaugh, J. H., Griffin, T. B., and Oates, J. A. (1970) Effect of amphetamine on the pressor response to tyramine. Formulation of p-hydroxynorephedrine from amphetamine in man. Clin. Pharmacol. Therap., 11: 656.

Cession-Fossion, A. (1970) Sur quelques propriétés pharmacologiques du fenproporex chez le rat. Arch. Int. Pharmacodyn., 187: 192-198.

Chattaway, F. D. (1931) Acetylation in aqueous alkaline solution. J. Chem. Soc. (Lond.), Part I: 2495-2496.

Cho, A. K., Hodshon, B. J., Lindeke, B., and Jonsson, J. (1975) The p-hydroxylation of amphetamine and phentermine by rat liver microsomes. Xenobiotica, 5: 531-538.

Cho, A. K., Lindeke, B., Hodshon, B. J., and Jenden, D. J. (1973) Deuterium substituted amphetamine as an internal standard in a gas chromatographic-mass spectrometric (GC-MS) assay for amphetamine. Anal. Chem., 45: 570-574.

Christian, S. T., Benington, F., Morin, R. D., and Corbett, L. (1975) Gas-liquid chromatographic separation and identification of biologically important indolealkylamines from human cerebrospinal fluid. Biochem. Med., 14: 191-200.

Clark, C. C. (1975) Determination of amphetamine by Schiff base formation and quantitative gas-liquid chromatography. J. Assoc. Off. Anal. Chem., 58: 1174-1177.

Clark, C. R., Teague, J. D., Wells, M. M., and Ellis, J. H. (1977) Gas and high pressure liquid chromatographic properties of some 4-nitrobenzamides of amphetamines and related arylalkylamines. Anal. Chem., 49: 912-915.

Clarke, D. D., Wilk, S., Gitlow, S. E., and Franklin, M. J. (1967) Gas chromatographic determination of dopamine at nanogram level. J. Gas Chromatogr., 5: 307.

Clarke, D. D., Wilk, S., and Gitlow, S. E. (1966) Electron-capture properties of halogenated amine derivatives. J. Gas Chromatogr., 4: 310.

Cockerill, A. F., Mallen, D. N. B., Osborne, D. J., and Prime, D. M. (1975) Analysis of 1-(2-phenyladamant-1-yl)-2-methylaminopropane as its chlorodifluoroacetyl derivative. J. Chromatogr., 114: 151-158.

Cohen, S., Vzan, A., and Valette, G. (1962) Thiāmine et dithiopropylthiāmine: étude de leur métabolisme par marquage au soufre 35 chez la souris et le rat. Biochem. Pharmacol., 11: 721-732.

Contractor, S. F. (1966) A rapid quantitative method for the estimation of 5-hydroxyindoleacetic acid in human urine. Biochem. Pharmacol., 15: 1701-1706.

Coutts, R. T. and Baker, G. B. (1982) Gas chromatography, in Handbook of Neurochemistry, vol. 2 (Lajtha, A., ed.), pp. 429-448. Plenum Press, New York.

Coutts, R. T., Baker, G. B., and Calverley, D. G. (1980b) A rapid, sensitive method of measuring meta- and para-tyramine levels in urine using electron-capture gas chromatography. Res. Commun. Chem. Pathol. Pharmacol., 28: 177-184.

Coutts, R. T., Baker, G. B., LeGatt, D. F., and Pasutto, F. M. (1980c) A novel gas chromatographic method for analysis of phenolic amines in urine. Proc. World Conf. Clin. Pharmacol. Therap., London, U.K., Abstr. no. 0248.

Coutts, R. T., Baker, G. B., Pasutto, F. M., Liu, S.-F., LeGatt, D. F., and Prelusky, D. B. (1984a) Mass spectral analysis of perfluoroacylated derivatives of some arylalkylamines of biological interest. Biomed. Mass Spec., in press.

Coutts, R. T. and Beckett, A. H. (1977) Metabolic N-oxidation of primary and secondary aliphatic medicinal amines. Drug Metab. Rev., 6: 51-104.

Coutts, R. T. and Dawson, G. W. (1977) Urinary excretion of phenolic metabolites of N-(n-propyl)amphetamine in man. Res. Commun. Chem. Pathol. Pharmacol., 17: 349-352.

Coutts, R. T., Dawson, G. W., and Beckett, A. H. (1976a) In vitro metabolism of 1-phenyl-2-(n-propylamino)propane (N-propylamphetamine) by rat liver homogenates. J. Pharm. Pharmac., 28: 815-821.

Coutts, R. T., Dawson, G. W., and Jones, G. R. (1978a) In vivo metabolism of N-alkylamphetamines in the rat--the effect of N-alkyl chain length on oxidation of the aromatic ring. Res. Commun. Chem. Pathol. Pharmacol., 22: 589-592.

Coutts, R. T., Dawson, G. W., Kazakoff, C. W., and Wong, J. Y. (1976b) In vivo phenolic metabolites of N-alkylamphetamines in the rat. Evidence in favour of catechol formation. Drug Metab. and Disp., 4: 256-261.

Coutts, R. T., Foster, B. C., Jones, G. R., and Myers, G. E. (1979) Metabolism of (±)-N-(n-propyl)-amphetamine by Cunninghamella echinulata. Appl. Environ. Microbiol., 37: 429-432.

Coutts, R. T. and Jones, G. R. (1982) In vivo and in vitro O-methylation of 1-(3,4-dihydroxyphenyl)-2-(n-propylamino)propane--an intermediate in N-(n-propyl) amphetamine metabolism. Res. Commun. Chem. Pathol. Pharmacol., 36: 173-176.

Coutts, R. T., Jones, G. R., and Liu, S.-F. (1982) Metabolism of N-alkylating derivatives of amphetamine by guinea pig and rabbit liver homogenates. Proc. West. Pharmacol. Soc., 25: 119-123.

Coutts, R. T., Jones, G. R., and Townsend, R. E. (1978b) Species differences in the in vitro metabolic reduction of the amphetamine metabolite, 1-phenyl-2-propanone. J. Pharm. Pharmac., 30: 415-418.

Coutts, R. T., Liu, S.-F., Hargesheimer, E. E., and Pasutto, F. M. (1980a) Studies on the isolation and quantitation of hydroxylated amines in aqueous solution. Can. J. Neurol. Sci., 7: 236.

Coutts, R. T., Nazarali, A. J., Baker, G. B., Micetich, R. G., and Hall, T. W. (1983) N-2-Cyanoethyl analogue of tranylcypromine: studies in rat brain. Proc. 43rd International Congress of Pharmaceutical Sciences of Federation Internationale Pharmaceuticale, p. 108.

Coutts, R. T., Prelusky, D. B., and Baker, G. B. (1984b) Determination of amphetamine, norephedrine and their phenolic metabolites in rat brain by gas-liquid chromatography. J. Pharm. Sci., in press.

Cox, R. H. Jr. and Maickel, R. P. (1972) Comparison of anorexigenic and behavioural potency of phenylethylamines. J. Pharmacol. Exptl. Therap., 181: 1-9.

Cox, R. H. Jr. and Perhach, J. L. Jr. (1973) A sensitive, rapid and simple method for the simultaneous spectrophotofluorometric determinations of norepinephrine, dopamine, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in discrete areas of brain. J. Neurochem., 20: 1777-1780.

Coyle, J. T. and Snyder, S. H. (1969) Catecholamine uptake by synaptosomes in homogenates of rat brain: stereospecificity in different areas. J. Pharmacol. Exptl. Therap., 170: 221-231.

Creveling, C. R., Daly, J. W., Tokuyama, T., and Witkop, B. (1969) Labile lipophilic derivatives of norepinephrine capable of crossing the blood-brain barrier. Experientia, 25: 26-27.

Cristofoli, W. A., Baker, G. B., and Coutts, R. T. (1982a) Amphetamine in rat brain and urine after administration of pheniprazine. Proc. West. Pharmacol. Soc., 25: 129-131.

Cristofoli, W. A., Baker, G. B., Coutts, R. T., and Benderly, A. (1982b) Analysis of a monofluorinated analogue of amphetamine in brain tissue using gas chromatography with electron-capture detection. Prog. Neuropsychopharmacol. & Biol. Psychiat., 6: 373-376.

Cummings, L. M. (1971) in Recent Advances in Gas Chromatography (Dombsky, I. and Perry, J., eds), p. 313. Marcel Dekker, New York.

Cummings, L. M. and Fourier, M. J. (1969) GLC determination of pseudoephedrine and related ephedrines in serum as the heptafluorobutyryl derivatives. Anal. Lett., 2: 403-409.

Daly, J. W., Creveling, C. R., and Witkop, B. (1966) The chemo-release of norepinephrine from mouse hearts. Structure-activity relationships. I. Sympathomimetic and related amines. J. Med. Chem., 9: 273-279.

Daly, J., Inscoe, J. K., and Axelrod, J. (1965) The formation of O-methylated catechols by microsomal hydroxylation and subsequent enzymatic catechol-O-methylation. Substrate specificity. J. Med. Chem., 8: 153-157.

Danielson, T. J. and Boulton, A. A. (1974) Detection and quantitative analysis of amphetamine. Biomed. Mass Spect., 1: 159-162.

Danielson, T. J. and Boulton, A. A. (1976) Distribution and occurrence of amphetamine and p-hydroxyamphetamine in tissues of the rat after injection of d-amphetamine sulfate. Eur. J. Pharmacol., 37: 257-264.

Decker, W. J. and Thompson, J. D. (1978) Rapid detection of amphetamine in urine by micro thin-layer chromatography and fluorescence. Clin. Toxicol., 13: 545-549.

Decroix, G. A. R., Gobert, J. G., and de Deurwaerder, R. (1968) Gas chromatographic method for the determination of glycerol in incubates of adipose tissues. Anal. Biochem., 25: 523-531.

Degen, P. H., DoAmaral, J. R., and Barchas, J. D. (1972) A gas-liquid chromatographic assay of melatonin and indoleamines using heptafluorobutyryl derivatives. Anal. Biochem., 45: 634-644.

Dehennin, L., Reiffsteck, A., and Scholler, R. (1972) Halogenated diesters of testosterone. Response to electron-capture detection, gas chromatographic and mass spectrometric properties. J. Chromatogr. Sci., 10: 224-230.

Dekirmenjian, H. and Maas, J. W. (1970) An improved procedure for 3-methoxy-4-hydroxyphenylethylene glycol determination by gas-liquid chromatography. Anal. Biochem., 35: 113-122.

Delbeke, F. T. and Debackere, M. (1977) Detection of sympathomimetic central nervous stimulants with special reference to doping. I. Comparative study of a conventional extraction procedure and adsorption chromatography using XAD-2 resin. J. Chromatogr., 133: 214-217.

Dewhurst, W. G. (1961) Amine metabolism in depressive illness with deductions on function drawn from other clinical data. Thesis, Univ. of London.

Dewhurst, W. G. (1970) The blood-brain barrier and other membrane phenomena in psychopharmacology, in Principles of Psychopharmacology (Clark, W. G. and del Giudice, J., eds), pp. 105-112. Academic Press, New York.

Dewhurst, W. G. (1968) New theory of cerebral amine function and its clinical application. Nature (Lond.), 218: 1130-1133.

Dewhurst, W. G. (1965) On the chemical basis of mood. J. Psychosom. Res., 9: 115-127.

Dewhurst, W. G. and Marley, E. (1965) Action of sympathomimetic and allied amines on the central nervous system of the chicken. Brit. J. Pharmacol., 25: 705-727.

Dewhurst, W. G. and McKim, H. R. (1980) Pharmacological effects of p-chloroamphetamine with respect to current amine hypotheses of affective disorders. Neuropsychobiology, 6: 66-71.

Digregorio, G. J. and Kniaz, E. K. (1976) Radioimmunoassay of amphetamines in rat parotid saliva. Drug Alcohol Depend., 1: 377-382.

Donike, M. (1973) Acylierung mit bis (acylamiden) N-methyl-bis (trifluoroacetamid) und bis (trifluoracetamid), zwei neue reagenzien zur trifluoracetylierung. J. Chromatogr., 78: 273-279.

Downer, R. G. H., Bailey, B. A., and Martin, R. J. (1984) Estimation of biogenic amines by HPLC and electrochemical detection. Chromatogr. Rev., 11: 5-7.

Dren, A. T., Jochimsen, W. G., and Plotnikoff, N. P. (1971) Comparison of pemoline, cocaine, methamphetamine and methylphenidate self-administration in monkeys. Pharmacologist, 13: 281.

Dren, A. T., Jochimsen, W. G., and Plotnikoff, N. P. (1972) Comparison of pemoline with other psychostimulants as reinforcers of self-administration behaviour in rhesus monkeys. Pharmacologist, 14: 59.

Dreyfus, J., Ross, J. J. Jr., and Schreiber, E. C. (1971) Excretion and biotransformation of the enanthate ester of fluphenazine-^{14}C by the dog. J. Pharm. Sci., 60: 829-833.

Driscoll, R. C., Barr, F. S., Gragg, B. J., and Moore, G. W. (1971) Determination of therapeutic blood levels of methamphetamine and pentobarbital by G.C. J. Pharm. Sci., 60: 1492-1495.

Drozd, J. (1975) Chemical derivatization in gas chromatography. J. Chromatogr., 113: 303-356.

Durden, D. A. and Philips, S. R. (1980) Kinetic measurements of the turnover rates of phenylethylamine and tryptamine in vivo in the rat brain. J. Neurochem., 34: 1725-1732.

Durden, D. A., Philips, S. R., and Boulton, A. A. (1973) Identification and distribution of β-phenylethylamine in the rat. Can. J. Biochem., 51: 995-1002.

Dyck, L. E. (1983) Release of monoamines from striatal slices by phenelzine and β-phenylethylamine. Prog. Neuro-Psychopharmacol. & Biol. Psychiat., 7: 797-800.

Earley, C. J. and Leonard, B. E. (1978) Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine, and several metabolites from brain tissue using disposable Bio-rad columns packed with Sephadex G-10. J. Pharmacol. Methods, 1: 67-79.

Ebert, A. G. and Hess, S. M. (1965) The distribution and metabolism of fluphenazine enanthate. J. Pharmacol. Exptl. Therap., 148: 412-421.

Edwards, D. J. and Blau, K. (1972) Analysis of phenylethylamines in biological tissues by gas-liquid chromatography with electron-capture detection. Anal. Biochem., 45: 387-402.

Edwards, D. J., Doshi, P. S., and Hanin, I. (1979) Analysis of phenylethylamines by gas chromatography-chemical ionization mass spectrometry. Anal. Biochem., 96: 308-316.

Ellinwood, E. H. (1967) Amphetamine psychosis. I. Description of the individuals and process. J. Nerv. Mental Dis., 144: 273-283.

Ellinwood, E. H. (1968) Amphetamine psychosis. II. Theoretical considerations. Int. J. Neuropsychiat., 4: 45-54.

Faivre, G., Dodinot, B., and Hua, G. (1969) Traitement de l'obésité chez le cardiaques expérimentation d'un nouvel anorexigène. La Vie Medicale, 49: 730.

Farrell, B. M. and Jefferies, T. M. (1983) An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines. J. Chromatogr., 272: 111-128.

Feenstra, M. G. P., Homan, J. W., Dijkstra, D., Mulder, T. B. A., Rollema, H., Westerink, B. H. C., and Horn, A. S. (1982) Reversed-phase liquid chromatography with amperometric detection of lipophilic dopamine analogues and determination of brain and serum concentrations after sample clean-up on small Sephadex G-10 columns. J. Chromatogr., 230: 271-287.

Fellows, L., Riederer, P., and Sandler, M. (1975) A rapid assay of 4-hydroxy-3-methoxyphenylglycol in urine. Clin. Chim. Acta, 59: 255-257.

Fenimore, D. C., Whitford, J. H., Davies, D. M., and Zlatkis, A. (1977) Nickel gas chromatographic columns: an alternative to glass for biological samples. J. Chromatogr., 140: 9-15.

Finkelstein, J., Chiang, E., and Lee, J. (1965) Synthesis of cis- and trans-2-phenoxycyclopropylamines and related compounds. J. Med. Chem., 8: 432-439.

Fischer, E. and Heller, B. (1972) Phenylethylamine as a neurohumoral agent in brain. Behav. Neuropsychiatr., 4: 8-11.

Francis, A. J., Morgan, E. D., and Poole, C. F. (1978) Flophemesyl derivatives of alcohols, phenols, amines and carboxylic acids and their use in gas chromatography with electron-capture detection. J. Chromatogr., 161: 111-117.

Freed, C. R., Weinkam, R. J., Melmon, K. L., and Castagnoli, N. (1977) Chemical ionization mass spectrometric measurement of α-methyldopa and s-dopa metabolites in rat brain regions. Anal. Biochem., 78: 319-332.

Fuentes, J. A., Oleshansky, M. A., and Neff, N. H. (1975) A sensitive enzymatic assay for dextro- or levo-tranylcypromine in brain. Biochem. Pharmacol., 24: 1971-1973.

Fuentes, J. A., Oleshansky, M. A., and Neff, N. H. (1976) Comparison of the apparent antidepressant activity of (-) and (+) tranylcypromine in an animal model. Biochem. Pharmacol., 25: 801-804.

Fujihara, S., Nakashima, T., and Kurogochi, Y. (1983) Determination of polyamines in human blood by electron-capture gas-liquid chromatography. J. Chromatogr., 277, 53-60.

Fuller, R. W. (1972) Selective inhibition of monoamine oxidase, in Advances in Biochemical Psychopharmacology, vol. 5 (Costa, E. and Sandler, M., eds), pp. 339-354. Raven Press, New York.

Fuller, R. W., Perry, K. W., and Molloy, B. B. (1974) Effect of an uptake inhibitor on serotonin metabolism in rat brain: studies with 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenyl propylamine (Lilly 110140). Life Sci., 15: 1161-1171.

Fuller, R. W. and Wong, D. T. (1984) Fluoxetine, a selective inhibitor of serotonin uptake in animal studies, in Clinical Neuropharmacology, vol. 7, suppl. 1; 14th Collegium Internationale Neuro-Psychopharmacologicum, S 106, pp. 202-203. Raven Press, New York.

Gal, J. (1977) Stereochemistry of metabolism of amphetamines: use of (-)-alpha-methoxy-alpha-(trifluoromethyl) phenylacetyl chloride for GLC resolution of chiral amines. J. Pharm. Sci., 66: 169-172.

Gelpi, E. (1982) Gas chromatography-mass spectrometry and selected ion monitoring of biogenic amines and related metabolites, in Analysis of Biogenic Amines (Baker, G. B. and Coutts, R. T., eds), pp. 151-181. Elsevier, Amsterdam.

Gelpi, E., Paralta, E., and Segura, J. (1974) Gas chromatography-mass spectrometry of catecholamines and tryptamines: determination of gas chromatographic profiles of the amines, their precursors and their metabolites. J. Chromatogr. Sci., 12: 701-709.

Generali, J. A., Hogan, L. C., McFarlane, M., Schwabb, S., and Hartman, C. R. (1981) Hypertensive crisis resulting from avocados and a MAO inhibitor. Drug Intell. Clin. Pharm., 15: 904-906.

Gil, R., Bal, T. S., and Moffat, A. C. (1982) The application of derivative UV-visible spectroscopy in forensic toxicology. J. Forensic Sci. Soc., 22: 165-171.

Goldstein, M. and Contrera, J. F. (1962) The substrate specificity of dopamine-β-hydroxylase. J. Biol. Chem., 237: 1898.

Gordis, C. and Neff, N. H. (1971) Monoamine oxidase: an approximation of turnover rates. J. Neurochem., 18: 1673-1682.

Gordon, E. K. and Oliver, J. (1971) 3-Methoxy-4-hydroxyphenylethylene glycol in human cerebrospinal fluid. Clin. Chim. Acta., 35: 145-150.

Gorrod, J. W. (1978) Extra-hepatic metabolism of drugs, in Drug Metabolism in Man (Gorrod, J. W. and Beckett, A. H., eds), pp. 157-174. Taylor and Francis Ltd., London.

Grahame-Smith, D. G. (1971) Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis, and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J. Neurochem., 18: 1053.

Green, A. R., Mitchell, B. D., Tordoff, A. F., and Youdim, M. B. H. (1977) Evidence for dopamine deamination by both type A and type B monoamine oxidase in rat brain in vivo, and for the degree of inhibition of enzyme necessary for increased functional activity of dopamine and 5-hydroxytryptamine. Brit. J. Pharmacol., 60: 343-349.

Greengard, P. (1975) Water soluble vitamins, in The Pharmacological Basis of Therapeutics (Goodman, L. S. and Gilman, A., eds), pp. 1549-1552. MacMillan, New York.

Griffith, J., Cavanaugh, J., and Oates, J. (1968) Paranoid psychosis in man induced by administration of d-amphetamine. Pharmacologist, 10: 180.

Hagopian, M., Dorfman, R. I., and Gut, M. (1961) A method for the isolation and separation of catecholamines and their transformation products from biological media. Anal. Biochem., 2: 387-390.

Hammel, I., Naot, Y., Ben-David, E., and Ginsburg, H. (1978) A simplified microassay for serotonin: modification of the enzymatic isotopic assay. Anal. Biochem., 90: 840-843.

Hampson, D. R. (1984) The monoamine oxidase inhibitor tranylcypromine: a comprehensive neurochemical study. Ph.D. thesis, Univ. of Alberta, Edmonton, Alberta.

Hampson, D. R., Baker, G. B., and Coutts, R. T. (1984a) A rapid and sensitive gas-chromatographic method for quantitation of 2-phenylethylamine in brain tissue and urine. Res. Commun. Chem. Pathol. Pharmacol., 43: 169-172.

Hampson, D. R., Baker, G. B., Nazarali, A. J., and Coutts, R. T. (1984b) A rapid and sensitive electron-capture gas chromatographic method for the analysis of tranylcypromine in brain tissue using acetylation and pentafluorobenzoylation. J. Biochem. Biophys. Methods, 9: 85-87.

Hansch, C., Steward, A. R., Anderson, S. M., and Bentley, D. (1968) The parabolic dependence of drug action upon lipophilic character as revealed by a study of hypnotics. J. Med. Chem., 11: 1-11.

Hauger, R. L., Skolnick, P., and Paul, S. M. (1982) Specific [^{3}H] β-phenylethylamine binding sites in rat brain. Eur. J. Pharmacol., 83: 147-148.

Hayes, T. S. (1973) Automated fluorometric determination of amphetamine in urine. Clin. Chem., 19: 390-394.

Hertel, G. and Fallot-Burghardt, W. (1978) Treatment of obese female patients with fenproporex within the framework of gynecologic practice. Fortschr. Med., 96: 2380-2382.

Hiemke, C., Kauert, G., and Kalbhen, D. A. (1978) Gas-liquid chromatographic properties of catecholamines, phenylethylamines, and indolealkylamines as their propionyl derivatives. J. Chromatogr., 153: 451-460.

Higa, S., Suzuki, T., Hayashi, A., Tsuge, I., and Yamamura, Y. (1977) Isolation of catecholamines in biological fluids by boric acid gel. Anal. Biochem., 77: 18-24.

Hirom, P. C. and Smith, R. L. (1978) in Isolation and Identification of Drugs (Clarke, E. G. C., ed.). Pharmaceutical Press, London, 2: 979-993.

Hoffman, A. R., Sastry, B. V. R., and Axelrod, J. (1979) Formation of α-methyldopamine ("catecholamphetamine") from *p*-hydroxyamphetamine by rat brain microsomes. Pharmacology, 19: 256-260.

Hollis, O. L. (1966) Separation of gaseous mixtures using porous polyaromatic polymer beads. Anal. Chem., 38: 309-316.

Hornykiewicz, O. (1966) Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Rev., 18: 925-964.

Hornykiewicz, O. (1984) Biochemical-pharmacological basis for new treatment strategies in Parkinson's disease, in Clinical Neuropharmacology, vol. 7, suppl. 1; 14th Collegium Internationale Neuro-Psychopharmacologicum, S 505, pp. 932-933. Raven Press, New York.

Imai, K., Sugiura, M., and Tamura, Z. (1971) Catecholamines in rat tissues and serum determined by gas chromatographic method. Chem. Pharm. Bull., 19: 409-411.

Jain, N. C. (1975) Rapid mass screening and confirmation of urinary amphetamine and methamphetamine by gas chromatography. Clin. Toxicol., 8: 211-224.

Jeanniot, J. Ph., Houin, G., Ledvdal, P., Berthet, D., Lusseav, D., Gross, P., and Tillement, J. P. (1983) Comparison of gas chromatographic-electron capture detection and high performance liquid chromatography for the determination of Butofilolol in biological fluids. J. Chromatogr., 278: 301-309.

Jones, R. S. G. (1981) In vivo pharmacological studies on the interactions between tryptamine and 5-hydroxytryptamine. Brit. J. Pharmacol., 73: 485-493.

Jones, R. S. G. and Boulton, A. A. (1980a) Tryptamine and 5-hydroxytryptamine: actions and interactions on cortical neurons in the rat. Life Sci., 27: 1849-1856.

Jones, R. S. G. and Boulton, A. A. (1980b) Interactions between p-tyramine, m-tyramine, or β-phenylethylamine and dopamine on single neurons in the cortex and caudate nucleus of the rat. Can. J. Physiol. Pharmacol., 58: 222-227.

Jonsson, J. A. (1974) Hydroxylation of amphetamine to para-hydroxyamphetamine by rat liver microsomes. Biochem. Pharmacol., 23: 3191-3197.

Jorgensen, A., Overo, K. F., and Hansen, V. (1971) Metabolism, distribution and excretion of flupenthixol decanoate in dogs and rats. Acta Pharmacol. Toxicol., 29: 339-359.

Jori, A. and Caccia, S. (1974) Distribution of amphetamine and its hydroxylated metabolites in various areas of the rat brain. J. Pharm. Pharmacol., 24: 746-748.

Jori, A., Caccia, S., Guiso, A., Ballabio, M., Garattini, S. (1979) Selective storage of p-hydroxy d-amphetamine in the dopaminergic nerve terminals. Biochem. Pharmacol., 28: 1205-1207.

Joseph, M. H. (1978) Determination of kynurenine by a simple gas-liquid chromatographic method applicable to urine, plasma, brain and cerebrospinal fluid. J. Chromatogr., 146: 33-41.

Julou, L., Bourat, G., Ducrot, R., Fournel, J., and Garrett, C. (1973) Pharmacological study of pipotiazine (19.366 R.P.) and its undecylenic (19.551 R.P.) and palmitic (19.552 R.P.) esters. Acta Psychiat. Scand., 241: 9-30.

Kaistha, K. K. and Jaffe, J. H. (1972) TLC techniques for identification of narcotics, barbiturates and CNS stimulants in a drug abuse urine screening program. J. Pharm. Sci., 61: 679-689.

Karasawa, T., Furukawa, K., Yoshida, K., and Shimizu, M. (1975) A double column procedure for the simultaneous estimation of norepinephrine, normetanephrine, dopamine, 3-methoxytyramine and 5-hydroxytryptamine in brain tissue. Japan. J. Pharmacol., 25: 727-736.

Karoum, F. (1984) Mass fragmentography as a tool in studies of central and peripheral biogenic amines, in Dynamics of Neurotransmitter Function (Hanin, I., ed.), pp. 357-362. Raven Press, New York.

Karoum, F., Cattabeni, F., Costa, E., Ruthven, C. R. J., and Sandler, M. (1972) Gas chromatographic assay of picomole concentrations of biogenic amines. Anal. Biochem., 47: 550-561.

Karoum, F., Ruthven, C. R. J., and Sandler, M. (1971) Gas chromatographic assay of phenolic alcohols in biological material using electron-capture detection. Biochem. Med., 5: 505-514.

Kawai, S. and Tamura, Z. (1968a) Gas chromatography of catecholamines as their trifluoroacetates. Chem. Pharm. Bull., 16: 699-701.

Kawai, S. and Tamura, Z. (1968b) Gas chromatography of catecholamines as their trifluoroacetates in urine and tumour. Chem. Pharm. Bull., 16: 1091-1094.

Kawasaki, C. (1963) Modified thiamine compounds. Vitam. & Horm., 21: 69-111.

Kinberger, B. (1981) Sensitive method for the high-performance liquid chromatographic determination of amphetamine in urinary extracts. J. Chromatogr., 213: 166-169.

Knapp, D. R. (1979) Handbook of Analytical Derivatization Reactions, pp. 65-145. John Wiley & Sons, New York.

Knights, A., Okasha, M. S., Salih, M. A., and Hirsch, S. R. (1979) Depressive and extrapyramidal symptoms and clinical effects: a trial of fluphenazine versus flupenthixol in maintenance of schizophrenic out-patients. Brit. J. Psychiat., 135: 515-523.

Knoll, J. and Magyar, K. (1972) Some puzzling pharmacological effects of monoamine oxidase inhibitors, in Advances in Biochemical Pharmacology, vol. 5 (Costa, E. and Sandler, M., eds), pp. 393-408. Raven Press, New York.

Ko, H., Lahti, R. A., Duchamp, D. J., and Royer, M. E. (1974) A GC-MS procedure for the measurement of dopamine in mouse striatal tissue. Anal. Lett., 7: 243-255.

Ko, H. and Petzold, E. N. (1978) Isolation of samples prior to chromatography, in GLC and HPLC Determination of Therapeutic Agents (Tsuji, K. and Moroyowich, W., eds), pp. 277-301. Marcel Dekker, New York.

Kojima, T., Une, I., and Yashiki, M. (1983) CI-Mass fragmentographic analysis of methamphetamine and amphetamine in human autopsy tissues after acute methamphetamine poisoning. Forensic Sci. Int., 21: 253-258.

Kreuz, D. S. and Axelrod, J. (1974) Amphetamine in human plasma: a sensitive and specific enzymatic assay. Science, 183: 420-421.

Krueger, G. L. and McGrath, W. R. (1964) 2-Benzylpiperidines and related compounds, in Psychopharmacological Agents, vol. 1 (Gordon, M., ed.), pp. 225-250. Academic Press, New York.

Kuhn, C. M., Schanberg, S. M., and Breese, G. R. (1978) Metabolism of amphetamine by rat brain tissue. Biochem. Pharmacol., 27: 343-351.

Kullberg, M. P. and Gorodetzky, C. W. (1974) Studies on the use of XAD-2 resin for detection of abused drugs in urine. Clin. Chem., 20: 177-183.

Kupchan, S. M. and Isenberg, A. C. (1967) Drug latentiation. III. Labile amide derivatives of normeperidine. J. Med. Chem., 10: 960-961.

Kuprys, R. and Oltmans, G. A. (1982) Amphetamine anorexia and hypothalamic catecholamines in genetically obese mice. Pharmacol. Biochem. Behav., 17: 271-282.

Lader, M. (1980a) Neurotransmitters, in Introduction to Psychopharmacology, pp. 12-29. Upjohn, Michigan.

Lader, M. (1980b) Antipsychotic drugs, in Introduction to Psychopharmacology, pp. 51-65. Upjohn, Michigan.

Lai, C. M. and Mason, W. D. (1973) New compounds: synthesis of alkyl esters of D,L-dopa. J. Pharm. Sci., 62: 510-511.

Landowne, R. A. and Lipsky, S. R. (1963) The electron-capture spectrometry of haloacetates: a means of detecting ultramicro quantities of sterols by gas chromatography. Anal. Chem., 35: 532-535.

Laverty, R. and Sharman, D. F. (1965) The estimation of small quantities of 3,4-dihydroxyphenylethylamine in tissues. Brit. J. Pharmacol., 24: 538-548.

LeGatt, D. F., Baker, G. B., and Coutts, R. T. (1981) Simultaneous extraction and separation of trace amines of biological interest. J. Chromatogr. Biomed. Appl., 225: 301-308.

Lewander, T. (1968) Urinary excretion and tissue levels of catecholamines during chronic amphetamine intoxication. Psychopharmacologia, 13: 394.

Lindenbaum, A., Marcher, K., Wepierre, J., and Cohen, Y. (1975) Blood-brain barrier. II. Kinetics of distribution of ^{3}H-para-hydroxyamphetamine in brain structures after intravenous administration in the rat. Arch. Int. Pharmacodyn. Ther., 215: 168-176.

Locock, R. A., personal communication.

Lovecock, J. E. and Lipsky, S. R. (1960) Electron-affinity spectroscopy. A new method for the identification of functional groups in chemical compounds separated by gas-chromatography. J. Amer. Chem. Soc., 82: 431-441.

Luine, V. N. and Paden, C. M. (1982) Effects of monoamine oxidase inhibition on female sexual behaviour, serotonin levels and type A and B monoamine oxidase activity. Neuroendocrinology, 34: 245-251.

Maggs, R. J., Joynes, P. L., Davies, A. J., and Lovecock, J. E. (1971) The electron-capture detector--a new mode of operation. Anal. Chem., 43: 1966-1971.

Maitre, L., Delini-Stula, A., and Waldmeir, P. C. (1976) Relationship between the degree of monoamine oxidase inhibition and some psychopharmacological responses to monoamine oxidase inhibitors in rats, in Monoamine Oxidase and Its Inhibition, Ciba Foundation Symposium 39, pp. 247-270. Elsevier/Excerpta Medica/North-Holland, Amsterdam.

Marde, Y. and Ryhage, R. (1978) Negative-ion mass spectrometry of amphetamine congeners. Clin. Chem., 24: 1720-1723.

Martin, I. L. (1982) Analysis of biogenic amines using radioenzymatic procedures, in Analysis of Biogenic Amines (Baker, G. B. and Coutts, R. T., eds), pp. 183-202. Elsevier, Amsterdam.

Martin, I. L. and Ansell, G. B. (1973) A sensitive gas chromatographic procedure for the estimation of noradrenaline, dopamine and 5-hydroxytryptamine in rat brain. Biochem. Pharmacol., 22: 521-533.

Martin, I. L. and Baker, G. B. (1976) Procedural difficulties in the gas-liquid chromatographic assay of the arylalkylamines. J. Chromatogr., 123: 45-50.

Martin, I. L. and Baker, G. B. (1977) The gas-liquid chromatographic method for the estimation of 2-phenylethylamine in rat brain tissue. Biochem. Pharmacol., 26: 1513-1516.

Martin, I. L., Baker, G. B., and Coutts, R. T. (1984) Gas-chromatography with electron-capture detection for measurement of bioactive amines in tissues and body fluids, in Methodological Surveys in Biochemistry and Analysis, 14 (Reid, E., ed.), in press. Plenum Press, New York.

Martindale The Extra Pharmacopoeia (1982a), 28th ed. (Reynolds, J. E. F. and Prasad, A. B., eds), pp. 65-70. Pharmaceutical Press, London.

Martindale The Extra Pharmacopoeia (1982b), 28th ed. (Reynolds, J. E. F. and Prasad, A. B., eds), pp. 131-132. Pharmaceutical Press, London.

Martindale The Extra Pharmacopoeia (1982c), 28th ed. (Reynolds, J. E. F. and Prasad, A. B., eds), pp. 886-887. Pharmaceutical Press, London.

Martindale The Extra Pharmacopoeia (1982d), 28th ed. (Reynolds, J. E. F. and Prasad, A. B., eds), pp. 1529-1531. Pharmaceutical Press, London.

Martinez, D. and Gimenez, M. P. (1983) Determination of amphetamine and methylamphetamine by gas-liquid chromatography (head space). Hum. Toxicol., 2: 391-393.

Mason, P. A., Law, B., and Moffat, A. C. (1983) Amphetamine radioimmunoassay: chemical tuning of specificity. J. Immunoassay, 4: 83-89.

Masuoka, D. T., Alejando, F. A., and Schott, H. F. (1982) [^{3}H]Dopamine release by d-amphetamine from striatal synaptosomes of reserpinized rats. Biochem. Pharmacol, 31: 1969-1974.

Matin, S. B., Wan, S. H., and Knight, J. B. (1977) Quantitative determination of enantiomeric compounds. I. Simultaneous measurement of the optical isomers of amphetamine in human plasma and saliva using chemical ionization mass spectrometry. Biomed. Mass Spec., 4: 118-121.

Matin, S. B. and Rowland, M. (1972) Electron-capture sensitivity comparison of various derivatives of primary and secondary amines. J. Pharm. Sci., 61: 1235-1240.

Matsukawa, T., Yurugi, S., and Oka, Y. (1962) The synthesis of s-acylthiamine derivatives and their stability. Ann. N.Y. Acad. Sci., 98: 430-444.

Mawdsley, C. (1970) Treatment of Parkinsonism with laevo-dopa. Br. Med. J., 1: 331-337.

Mayer, S., Maickel, R. P., and Brodie, B. B. (1959) Kinetics of penetration of drugs and other foreign compounds into cerebrospinal fluid and brain. J. Pharmacol. Exptl. Therap., 127: 205-211.

McCallum, N. K. and Armstrong, R. J. (1973) The derivatisation of phenols for gas chromatography using electron-capture detection. J. Chromatogr., 78: 303-307.

McClure, D. A. (1975) The effect of a pro-drug of epinephrine (dipivalyl epinephrine) in glaucoma--general pharmacology, toxicology, and clinical experience, in Pro-Drugs as Novel Drug Delivery Systems (Higuchi, T. and Stella, V., eds), pp. 225-235. ACS Symposium, series 14.

McCown, T. J. and Barrett, R. J. (1980) Development of tolerance to the rewarding effects of self-administered s(+)-amphetamine. Pharmacol. Biochem. Behav., 12: 137-141.

McCullough, P. R. and Aue, W. A. (1973) "Temperature-programmed" gas chromatography with electron-capture detection. J. Chromatogr., 82: 269-278.

McGrath, W. R. and Kuhn, W. L. (1968) Pharmacologic effects of 2-phenylcycloalkylamines. Arch. Int. Pharmacodyn. Ther., 172: 405-413.

McNair, H. M. and Bonelli, E. J. (1969) Basic Gas Chromatography, pp. 12. Varian Aerograph, Walnut Creek, California.

Meola, J. M. and Vanko, M. (1974) Use of charcoal to concentrate drugs from urine before drug analysis. Clin. Chem., 20: 184-187.

Metha, A. C. and Schulman, S. G. (1974) Comparison of fluorometric procedures for assay of amphetamine. J. Pharm. Sci., 63: 1150-1151.

Midha, K. K., Cooper, J. K., Gagne, D., and Bailey, K. (1979a) Detection of nanogram levels of various ring substituted phenylisopropylamines in urine and plasma by GLC-ECD. J. Anal. Toxicol., 3: 53-58.

Midha, K. K., McGilveray, I. J., and Cooper, J. K. (1979b) A GLC-ECD assay for the simultaneous determination of fenfluramine and norfenfluramine in human plasma and urine. Can. J. Pharm. Sci., 14: 18-21.

Mitchard, M. (1978) Chromatographic methods in the study of drug metabolism in man, in Drug Metabolism in Man (Gorrod, J. W. and Beckett, A. H., eds), pp. 175-191. Taylor and Francis, London.

Miyazaki, H., Hashimoto, Y., Iwanaga, M., and Kubodera, T. (1974) Analysis of biogenic amines and their metabolites by gas chromatography chemical ionization mass spectrometry. J. Chromatogr., 99: 575-586.

Moffat, A. C. (1975) Use of SE-30 as a stationary phase for the gas-liquid chromatography of drugs. J. Chromatogr., 113: 69-95.

Moffat, A. C., Horning, E. C., Matin, S. B., and Rowland, M. (1972) Perfluorobenzene derivatives as derivatizing agents for the gas chromatography of primary and secondary amines using electron-capture detection. J. Chromatogr., 66: 255-260.

Montpetit, V. J. A., Andermann, F., Carpenter, S., Fawcett, J. S., Zborowska-Slvis, D., and Giberson, H. R. (1971) Subacute necrotizing encephalomyelopathy. A review and a study of two families. Brain, 94: 1-30.

Mule, S. J., Whitlock, E., and Jukofsky, D. (1975) Radioimmunoassay of drugs subject to abuse: critical evaluation of urinary morphine-barbiturate, morphine, barbiturate and amphetamine assays. Clin. Chem., 21: 81-86.

Murphy, D. L. (1972) Amine precursors, amines, and false neurotransmitters in depressed patients. Am. J. Psychiat., 129: 141-148.

Narasimhachari, N., Friedel, R. O., Schlemmer, F., and Davis, J. M. (1979) Quantitation of amphetamine in plasma and cerebrospinal fluid by gas chromatography-mass spectrometry-selected ion monitoring, using beta-methylphenylethylamine as internal standard. J. Chromatogr., 164: 386-393.

Narasimhachari, N. and Vouros, P. (1972) Gas-liquid chromatography and mass spectrometry of biogenic amines and amphetamines as their isothiocyanate derivatives. Anal. Biochem., 45: 154-163.

Nazarali, A. J., Baker, G. B., Coutts, R. T., and Pasutto, F. M. (1983) Amphetamine in rat brain after intraperitoneal injection of N-alkylated analogues. Prog. Neuropsychopharmacol. & Biol. Psychiat., 7: 813-816.

Nelson, L. M., Bubb, F. A., Lax, P. M., Weg, M. W., and Sandler, M. (1979) An improved method for the differential assay of 3-O-methylated catecholamines in human urine using ion-pair extraction and gas chromatography electron-capture detection. Clin. Chim. Acta, 92: 235-240.

Niwaguchi, T., Kanda, Y., Kishi, T., and Inoue, T. (1982) Determination of d-methamphetamine in urine after administration of d- or dl-methamphetamine to rats by radioimmunoassay using optically sensitive antiserum. J. Forensic Sci., 27: 592-597.

Nix, C. R. and Hume, A. S. (1970) A spectrophotofluorometric method for the determination of amphetamine. J. Forensic Sci., 15: 595-600.

Nogami, H., Hasegawa, J., and Noda, K. (1969a) Thiamine derivatives of disulfide type. I. Formation of thiamine from thiamine propyl disulfide in rat intestine _in vitro_. Chem. Pharm. Bull., 17: 219-227.

Nogami, H., Hasegawa, J., Nakatsuka, S., and Noda, K. (1969b) Thiamine derivatives of disulfide type. II. The formation of thiamine from the disulfide derivatives in rat intestine _in vitro_. Chem. Pharm. Bull., 17: 228-233.

Nogami, H., Hasegawa, J., and Noda, K. (1969c) Thiamine derivatives of disulfide type. III. Enzyme systems in rat intestine contributed for thiamine formation from the disulfide type derivatives. Chem. Pharm. Bull., 17: 234-241.

Nogami, H., Hasegawa, J., and Rikihisa, T. (1973) Thiamine derivatives of disulfide type. IV. The ring opening-closing reactions of 4-methylthiazolium salts. Chem. Pharm. Bull., 21: 858-866.

Noonan, J. S., Murdick, P. W., and Ray, R. S. (1969) A method for detecting amphetamine using gas chromatography of a halogenated derivative. J. Pharmacol. Exptl. Therap., 168: 205-209.

Nymark, M., Franck, K. F., Pedersen, V., Boeck, V., and Nielsen, I. M. (1973) Prolonged neuroleptic effect of α-flupenthixol decanoate in oil. Acta Pharmacol. Toxicol., 33: 363-376.

O'Brien, B. A., Bonicamp, J. M., and Jones, D. W. (1982) Differentiation of amphetamine and its major hallucinogenic derivatives using thin-layer chromatography. J. Anal. Toxicol., 6: 143-147.

Oldendorf, W. H. and Dewhurst, W. G. (1978) The blood-brain barrier and psychotropic drugs, in Principles of Psychopharmacology (Clark, W. G. and del Guidice, J., eds), pp. 183-191. Academic Press, New York.

Oliver, J. S., Smith, H., and Williams, D. J. (1977) The detection, identification and measurement of indole, tryptamine, and 2-phenylethylamine in putrefying human tissue. Forensic Sci., 9: 195-203.

Olsen, J., Vejlby, K., and Faurholt, C. (1952) Studies on carbamates. VII. The carbamates of n-propylamine and iso-propylamine. Acta Chem. Scand., 6: 398-403.

Olson, C. L. (1978) Theory and instrumentation, in GLC and HPLC Determination of Therapeutic Agents, Part 1 (Tsuji, K. and Moroyowich, W., eds), pp. 1-46. Marcel Dekker, New York.

Paul, S. M., Hulihan-Giblin, B., and Skolnick, P. (1982) (+)-Amphetamine binding to rat hypothalamus: relation to anorexic potency for phenylethylamines. Science, 218: 487-490.

Peaston, M. J. and Bianchine, J. R. (1970) Metabolic studies and clinical observations during L-dopa. Brit. Med. J., 1: 400-403.

Perry, J. A. and Feit, C. A. (1978) Derivatization techniques in gas-liquid chromatography, in GLC and HPLC Determination of Therapeutic Agents, Part 1 (Tsuji, K. and Moroyowich, W., eds), pp. 137-208. Marcel Dekker, New York.

Philips, S. R. and Boulton, A. A. (1979) The effect of monoamine oxidase inhibitors on some arylalkylamines in rat striatum. J. Neurochem., 33: 159-167.

Philips, S. R., Durden, D. A., and Boulton, A. A. (1974) Identification and distribution of tryptamine in the rat. Can. J. Biochem., 52: 447-451.

Pincus, J. H. (1972) Subacute necrotizing encephalomyelopathy (Leigh's disease): a consideration of clinical features and etiology. Develop. Med. Child. Neurol., 14: 87-101.

Pinder, R. M. (1970) Possible dopamine derivatives capable of crossing the blood-brain barrier in relation to Parkinsonism. Nature, 228: 358.

Plauchu, M. M., Arnaud, P., and Pousset, G. (1968) Étude clinique d'un nouvel anorexigène. Lyon Med., 14: 111.

Pletscher, A., Bartholini, G., Bruderer, H., Burkard, W. P., and Gey, K. F. (1964) Chlorinated arylalkylamines affecting the cerebral metabolism of 5-hydroxytryptamine. J. Pharmacol. Exptl. Therap., 145: 334-350.

Poole, C. E. and Morgan, E. D. (1975) Structural requirements for the electron-capturing properties of ecdysones. J. Chromatogr., 115: 587-590.

Portoghese, P. S., Pazdernik, T. L., Kuhn, W. L., Hite, G., and Shafi'ee, A. (1968) Stereochemical studies on medicinal agents. V. Synthesis, configuration and pharmacological activity of pipradrol enantiomers. J. Med. Chem., 11: 12-15.

Powers, K. H. and Ebert, M. H. (1979) Comparison of radioimmunoassay and gas chromatographic-mass spectrometric assay for d-amphetamine. Biomed. Mass Spect., 6: 187-190.

Prelusky, D. B. (1983) In vitro models for drug metabolism studies. Ph.D. thesis, Univ. of Alberta, Edmonton, Alberta.

Rafaelson, O. J. (1976) in Monoamine Oxidase and Its Inhibition, Ciba Foundation Symposium 39 (new series), pp. 294. Elsevier, Amsterdam.

Raiteri, M., Del Carmine, R., Bertollini, A., and Levi, G. (1977) Effect of sympathomimetic amines on the synaptosomal transport of noradrenaline, dopamine, and 5-hydroxytryptamine. Eur. J. Pharmacol., 41: 133-143.

Rao, T. S., personal communication.

Renaud, B., Quenin, P., and Quincy, C. (1974) Determination fluorimétrique en flux continu de l'acide homovanillique. Application au liquide céphalorachidien. Clin. Chim. Acta, 52: 179-185.

Reynolds, G. P. and Gray, D. O. (1976) A method for the estimation of 2-phenylethylamine in human urine by gas chromatography. Clin. Chim. Acta., 70: 213-217.

Reynolds, G. P., Riederer, P., Sandler, M., Jellinger, K., and Seeman, D. (1978) Amphetamine and 2-phenylethylamine in post-mortem Parkinsonian brain after (-) deprenyl administration. J. Neural Trans., 43: 271-277.

Rindi, G. and Ventura, U. (1972) Thiamine intestinal transport. Physiol. Rev., 52: 821-827.

Rivera-Calimlim, L., Dujovne, C. A., Morgan, J. P., Lasagna, L., and Bianchine, J. R. (1970) L-Dopa treatment failure: explanation and correction. Brit. Med. J., 4: 93-94.

Robinson, D. L., Nies, A., Ravaris, C. L., Ives, J. A., and Bartlett, D. (1978) Clinical pharmacology of phenelzine. Arch. Gen. Psychiat., 35: 629-638.

Roder, E. and Merzhauser, J. (1974) Determination of biogenic amines by high-pressure liquid chromatography. Anal. Chem., 34: 272.

Ross, S. B., Renyi, A. L., and Brunfelter, B. (1968) Cocaine sensitive uptake of sympathomimetic amines in nerve tissue. J. Pharm. Pharmacol., 20: 283-288.

Roth, J. A. and Gillis, C. N. (1975) Multiple forms of amine oxidase in perfused rabbit lung. J. Pharmacol. Exptl. Therap., 194: 537-544.

Saavedra, J. M. (1974) Enzymatic-isotopic assay for the presence of β-phenylethylamine in brain. J. Neurochem., 22: 211-216.

Saavedra, J. M. (1984) The use of enzymatic radioisotopic microassays for the quantification of β-phenylethylamine, phenylethanolamine, tyramine, and octopamine, in Neurobiology of the Trace Amines (Boulton, A. A., Baker, G. B., Dewhurst, W. G., and Sandler, M., eds), pp. 41-55. Humana Press, Clifton, N.J.

Sabelli, H. C., Fawcett, J., Gusovsky, F., Javaid, J., Edwards, J., and Jeffriess, H. (1983) Urinary phenyl acetate: a diagnostic test for depression? Science, 220: 1187-1188.

Sabelli, H. C. and Mosnaim, A. D. (1974) Phenylethylamine hypothesis of affective behaviour. Am. J. Psychiatry, 131: 695-699.

Sandler, M., Glover, V., Ashford, A., and Stern, G. M. (1978) Absence of "cheese effect" during deprenyl therapy: some recent studies. J. Neural Trans., 43: 209-215.

Sandler, M. and Reynolds, G. P. (1976) Does phenylethylamine cause schizophrenia? Lancet, 1: 70.

Sasa, S. and Blank, C. L. (1977) Determination of serotonin and dopamine in mouse brain tissue by high-performance liquid chromatography with electro-chemical detection. Anal. Chem., 49: 354-359.

Schuster, C. R., Woods, J. H., and Seevers, M. H. (1969) Self administration of central stimulants by the monkey, in Abuse of Central Stimulants (Sjoqvist, F. and Tottie, M., eds), pp. 339-347. Raven Press, New York.

Schweitzer, J. W. and Friedhoff, A. J. (1970) Amphetamines in human urine: rapid estimation by gas-liquid chromatography. Clin. Chem., 16: 786-788.

Seiler, K. U., Wassermann, O., and Wensky, H. (1976) On the role of serotonin in the pathogenesis of pulmonary hypertension induced by anorectic drugs; an experimental study in the isolated perfused rat lung. Clin. Exp. Pharmac. Physiol., 3: 323-330.

Sen, N. P. (1969) Analysis and significance of tyramine in food. J. Food Sci., 34: 22-26.

Sevcik, J. (1976) in Detectors in Gas Chromatography, vol. 4, pp. 72-86. Journal of Chromatography Library, Elsevier, Amsterdam.

Sever, P. S., Caldwell, J., and Williams, R. T. (1974) Evidence against the involvement of false neurotransmitters in tolerance to amphetamine-induced hyperthermia in the rat. J. Pharm. Pharmacol., 26: 823.

Sever, P. S., Caldwell, J., and Williams, R. T. (1976) Tolerance to amphetamine in two species (rat and guinea pig) that metabolize it differently. Psychol. Med., 6: 35-42.

Shafi'ee, A. and Hite, G. (1969) The absolute configuration of the pheniramines, methylphenidates and pipradrols. J. Med. Chem., 12: 266-270.

Shaw, F. W. (1938) The estimation of adrenaline. Biochem. J., 32: 19-25.

Shriner, R. L. and Child, R. G. (1952) The synthesis of N-substituted carbamates. J. Am. Chem. Soc., 74: 549-550.

Simpson, L. L. (1978) Blood pressure and heart rate responses produced by d-amphetamine: correlation with blood levels of drug. J. Pharmacol. Exptl. Therap., 205: 366-373.

Sioufi, A., Colussi, D., and Mangoni, P. (1983b) Gas chromatographic determination of oxprenolol in human plasma. J. Chromatogr., 278: 185-188.

Sioufi, A. and Pommier, F. (1983a) Determination of isosorbide as a metabolite of isosorbide dinitrate in human urine by capillary gas chromatography with electron-capture detection. J. Chromatogr., 277: 157-164.

Snodgrass, S. R. and Horn, A. S. (1973) An assay procedure for tryptamine in brain and spinal cord using its [^{3}H]-dansyl derivative. J. Neurochem., 21: 687-696.

Souter, R. W. (1975) Gas chromatographic resolution of enantiomeric amphetamines and related amines. II. Effects of cyclic structures on diastereomer and enantiomer resolution. J. Chromatogr., 114: 307-313.

Stella, V. (1975) Pro-drugs: an overview and definition, in Pro-Drugs as Novel Drug Delivery Systems (Higuchi, T., and Stella, V., eds), pp. 1-115. ACS Symposium, series 14.

Steranka, L. R. (1983) Long-term effects of a priming dose and short-term infusion of amphetamine on striatal dopamine neurons in rats. Eur. J. Pharmacol., 96: 159-163.

Stevens, H. M. (1973) A spectrophotometric method for screening urine samples for amines, including amphetamine and methylamphetamine. J. Forensic Sci. Soc., 13: 119-125.

Sugiura, M. and Hirano, K. (1974) Determination of prostaglandin F1 alpha and F2 alpha by gas-liquid chromatography. J. Chromatogr., 90: 169-177.

Suzuki, S. and Yagi, K. (1976) A fluorometric assay of β-phenylethylamine in rat brain. Anal. Biochem., 75: 192-200.

Tabakoff, B., Moses, F., Philips, S. R., and Boulton, A. A. (1977) Effects of tranylcypromine and pargyline on brain tryptamine. Experientia, 33: 380-381.

Taylor, K. M. and Snyder, S. H. (1970) Amphetamine: differentiation by d- and l-isomers of behaviour involving brain norepinephrine or dopamine. Science, 168: 1487-1489.

Taylor, W. A. and Sulser, F. (1973) Effects of amphetamine and its hydroxylated metabolites on central noradrenergic mechanisms. J. Pharmacol. Exptl. Therap., 185: 620-632.

Temple, D. M. and Gillespie, R. (1966) Liquid ion-exchange extraction of some physiologically active amines. Nature (Lond.), 209: 714-715.

Teotino, U. M., Della Bella, D., Gandini, A., and Benelli, G. (1967) Chemical and biological properties of some aminomethyl-2-phenylcyclopropane derivatives. Pharmacological comparison with tranylcypromine. J. Med. Chem., 10: 1091-1096.

Terada, M., Yamamoto, T., Yoshida, T., Kuroiwa, Y., and Yoshimura, S. (1982) Rapid and highly sensitive method for determination of methamphetamine and amphetamine in urine by electron-capture gas chromatography. J. Chromatogr., 237: 285-292.

Testa, B. and Salvesen, B. (1980) Quantitative structure-activity relationships in drug metabolism and disposition: pharmacokinetics of N-substituted amphetamines in humans. J. Pharm. Sci., 69: 497-501.

Thoenen, H., Hurlimann, A., and Haefely, W. (1968) Mechanism of amphetamine accumulation in the isolated perfused heart of the rat. J. Pharm. Pharmacol., 20: 1-11.

Thoenen, H., Hurlimann, A., Gey, K. F., and Haefely, W. (1966) Liberation of p-hydroxynorephedrine from cat spleen by sympathetic nerve stimulation after pretreatment with amphetamine. Life Sci., 5: 1715-1722.

Thomson, A. D., Baker, H., and Leevy, C. M. (1968) Thiamine absorption in alcoholism. Amer. J. Clin. Nutr., 21: 537-538.

Thomson, A. D., Baker, H., and Leevy, C. M. (1970) Patterns of ^{35}S-thiamine hydrochloride absorption in the malnourished alcoholic patient. J. Lab. Clin. Med., 76: 34-45.

Thomson, A. D., Frank, O., Baker, H., and Leevy, C. M. (1971) Thiamine propyl disulfide: absorption and utilization. Ann. Intern. Med., 74: 529-534.

Todoriki, H., Hayashi, T., Naruse, H., and Hirakawa, A. (1983) Sensitive high-performance liquid chromatographic determination of catecholamines in rat brain using a laser fluorimetric detection system. J. Chromatogr., 276: 45-54.

Tognoni, G., Morselli, P. L., and Garattini, S. (1972) Amphetamine concentrations in rat brain and human urine after fenproporex administration. Eur. J. Pharmacol., 20: 125-126.

Trinler, W. A., Reuland, D. J., and Hiatt, T. B. (1976) Screening of street drugs by high pressure liquid chromatography. II. The screening of some common amphetamines, ephedrine and phencyclidine by reverse-phase HPLC. J. Forensic Sci. Soc., 16: 133-138.

Turner, P., Young, J. H., and Paterson, J. (1967) Influence of urinary pH on the excretion of tranylcypromine sulfate. Nature, 215: 881-882.

Turner, P., Young, J. H., and Scowen, E. F. (1966) Fluorimetric detection of tranylcypromine in urine. J. Pharm. Pharmac., 18: 550-551.

Vague, J., Codaccioni, J. L., and Kelisbauer, J. P. (1967) Étude clinique chez 44 obèses d'un nouvel anorexigène actif par voie buccale: le fenproprex. Sud. Med. Chirurg., 2: 541.

Van der Schoot, J. B., Ariens, E. J., Van Rossum, J. M., and Hurkmans, J. A. (1961) Phenylisopropylamine derivatives, structure and action. Arzneim-Forschung, 9: 902-907.

Van Praag, H. M. and Korf, J. (1971) Endogenous depressions with and without disturbances in the 5-hydroxytryptamine metabolism: a biochemical classification? Psychopharmacology, 19: 148-152.

Verbiscar, A. J. and Abood, L. G. (1970) Carbamate ester latentiation of physiologically active amines. J. Med. Chem., 13: 1176-1179.

Vessman, J., Moss, A. M., Horning, M. G., and Horning, E. C. (1969) The GLC separation of indole amines and indole alcohols as heptafluorobutyryl derivatives. Anal. Lett., 2: 81-91.

Villeneuve, A. and Fontaine, P. (1980) A near decade experience with pipotiazine palmitate in chronic schizophrenia. Curr. Therap. Res., 27: 411-418.

Villeneuve, A., Pires, A., Jus, A., Lachance, R., and Drolet, A. (1972) A long-term pilot study of pipotiazine palmitate in chronic schizophrenia. Curr. Ther. Res., 14: 696-710.

Warembourg, H. and Jaillard, J. (1968) Expérimentation clinique due fenproporex dans le traitment des obésités. Lille Med., 13, Suppl.: 273.

Warsh, J. J., Chiu, A. S., and Godse, D. D. (1979) Determination of picogram levels of brain serotonin by a simplified liquid chromatographic electrochemical detection assay. Brain Res. Bull., 4: 567-570.

Warsh, J. J., Chiu, A. S., and Godse, D. D. (1982) Determination of biogenic amines and their metabolites by high-performance liquid chromatography, in Analysis of Biogenic Amines (Baker, G. B. and Coutts, R. T., eds), pp. 203-236. Elsevier, Amsterdam.

Warsh, J. J., Godse, D. D., Stancer, H. C., Chan, P. W., and Coscina, D. V. (1977) Brain tryptamine in rats by a new gas chromatography-mass fragmentographic method. Biochem. Med., 18: 10-20.

Westerink, B. H. C. (1984) Determination of normetanephrine, 3,4-dihydroxyphenylethyleneglycol (free and total), and 3-methoxy-4-hydroxyphenylethyleneglycol (free and total) in rat brain by high-performance liquid chromatography with electrochemical detection and effects of drugs on regional concentrations. J. Neurochem., 42: 934-942.

Westerink, B. H. C. and Korf, J. (1976) Regional rat brain levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid: concurrent fluorometric measurement and influence of drugs. Eur. J. Pharmacol., 38: 281-291.

Westerink, B. H. C. and Korf, J. (1977) Rapid concurrent automated fluorimetric assay of noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 3-methoxytyramine in milligram amounts of nervous tissue after isolation on Sephadex G-10. J. Neurochem., 29: 697-706.

Westerink, B. H. C. and Mulder, T. B. A. (1981) Determination of picomole amounts of dopamine, noradrenaline, 3,4-dihydroxyphenylalanine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid in nervous tissue after one-step purification on Sephadex G-10, using high-performance lqiuid chromatography with a novel type of electrochemical detection. J. Neurochem., 36: 1449-1462.

Wilk, S., Davis, K. L., and Thacker, S. B. (1971) Determination of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in cerebrospinal fluid. Anal. Biochem., 39: 498-504.

Wilkinson, G. R. (1970) The GLC separation of amphetamine and ephedrines as pentafluorobenzamide derivatives and their determination by electron-capture detection. Anal. Lett., 3: 289-298.

Williams, R. T., Caldwell, J., and Dring, L. G. (1973) Comparative metabolism of some amphetamines in various species, in Frontiers in Catecholamine Research (Usdin, E. and Snyder, S. H., eds), pp. 927-932. Pergamon Press, New York.

Windheuser, J. J. and Higuchi, T. (1962) Kinetics of thiamine hydrolysis. J. Pharm. Sci., 51: 354-364.

Wong, K. P., Ruthven, C. R. J., and Sandler, M. (1973) Gas chromatographic measurement of urinary catecholamines by an electron-capture detection procedure. Clin. Chim. Acta, 47: 215-222.

Wu, A. (1975) Rapid analysis of the central nervous system stimulants, amphetamines, via gas chromatography-mass spectrometry: rapid acylation in the presence of a mercury catalyst. Clin. Toxicol., 8: 225-232.

Wurtman, R. J. and Axelrod, J. (1963) A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol., 12: 1439-1440.

Youdim, M. B., Aaronson, J. K., Blau, K., Green, A. R., and Grahame-Smith, D. G. (1979) Tranylcypromine ("Parnate") overdose: measurement of tranylcypromine concentrations and MAO inhibitory activity and identification of amphetamines in plasma. Psychol. Med., 9: 377-382.

Zirkle, C. L. and Kaiser, C. (1964) Monoamine oxidase inhibitors (non-hydrazines), in Psychopharmacological Agents, vol. 1 (Gordon, M., ed.), pp. 445-554. Academic Press, New York.

Zirkle, C. L., Kaiser, C., Tedeschi, D. H., Tedeschi, R. E., and Burger, A. (1962) 2-Substituted cyclopropylamines. II. Effect of structure upon monoamine oxidase--inhibitory activity as measured in vivo by potentiation of tryptamine convulsions. J. Med. Pharm. Chem. 5: 1265-1284.

Zweig, G. and Sherma, J. (1972) in Handbook of Chromatography, vol. 2, pp. 1-23. Chemical Rubber Corp. Press, Cleveland, Ohio.